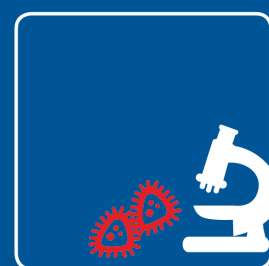


Thomas Thymann:

Ernæringsmæssige effekter af mælkefedt i mælkeerstatninger

Nutritional effects of dairy lipids in milk replacers



Final report

for collaborative projects funded via the Danish Dairy Research Foundation (DDRF)

1. Title of the project

Dansk: Ernæringsmæssige effekter af mælkefedt i mælkeerstatninger

English: Nutritional effects of dairy lipids in milk replacers

2. Project manager

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4. Sources of funding

The project is funded by the Milk Levy Fund, Novo Nordisk Fonden, Forskningsrådet for Teknologi og Produktion, and Mille Food

5. Project period

Projektperiode med MFF-finansiering: Jan 2020 - Dec 2022

6. Project summary

DANSK: Baggrund og Formål. Mælkeerstatninger til børn baserer sig på ingredienser af både animalsk og vegetabilsk oprindelse. Specifikt i forhold til mælkeerstatningernes fedt-fraktion, har man i en lang årrække benyttet vegetabiliske olier bla. for at øge indholdet af polyumættede fedtsyrer. Omvendt har fedt fra komælk mange ligheder til fedt i kvindemælk, og det er uklart om vegetabiliske olier reelt er bedre end fedt fra komælk. Det var projektets formål at bringe dokumentation for effekten af vegetabilisk fedt og mælkefedt på vigtige organer som hjerne, tarm, og immunitet. Det var endvidere vort formål at karakterisere fedtfordøjelsen hos nyfødte med særlige komplikationer, herunder lav fødselsvægt, tidlig fødsel eller manglende sekretion af galde. Projektet blev medfinansieret af Novo Nordisk Fonden, Forskningsrådet for Teknologi og Produktion samt Mille Food.

Resultater: Grise blev ernæret med mælkeerstatning de første tre uger efter fødsel, hvor fedt-delen enten stammede fra komælk eller vegetabiliske kilder. Resultaterne viste at vækst og hjerneudvikling er ens uanset fedttype. Blodets fedtprofil varierede væsentligt (som forventet), mens hjernefedtsammensætningen forblev mere stabil. Endvidere fandt vi at immunsystemet blev positivt påvirket af fedt fra komælk. I et andet studie etablerede vi en grisemodel med kirurgisk induceret kolestase, dvs manglende flow af galde fra leveren til tarmen, - en komplikation der ses hos visse nyfødte børn. Tilstanden ledte til leverforandringer og negative effekter på hjernen. Denne model muliggør fremtidige undersøgelser af ernærings- og lægemiddelinterventioner for patienter med nedsat fedtfordøjelse.

Konklusion: Samlet indikerer resultaterne at både vegetabilisk og bovint mælkefedt er egnede til mælkeerstatninger til nyfødte, uanset fødselsvægt. Vi så ingen forskel i hjerneudvikling indenfor den tre-ugers forsøgsperiode. Det kan dog ikke udelukkes at længerevarende studier vil lede til forskelle i hjerneudvikling. Immunsystemet blev positivt påvirket af bovint fedt ved at øge evnen til at nedbryde smitstoffer som *E.coli*. Denne immunologiske evne er vigtig, ikke mindst for nyfødte som er sårbare overfor infektioner. Slutteligt konkluderer vi at den udviklede kolestasemodel baner vejen for nye behandlingsmuligheder for spædbørn med galdevejsproblemer.

ENGLISH: Background and Purpose: Infant formula is based on ingredients of both animal and plant origin. Specifically, regarding the fat fraction of infant formula, vegetable oils have been used for many years to mimic the fatty acid profile of human milk as closely as possible. However, it remains unclear whether vegetable oils are actually better than fat from cow's milk. The aim of this project was to provide documentation on the effects of vegetable and dairy fat on key organs such as the brain, intestines, and immune system. Additionally, our goal was to characterize fat digestion in newborns with specific complications, including low birth weight, premature birth, or lack of bile secretion. The project was co-financed by the Novo Nordisk Foundation, the Research Council for Technology and Production, and Mille Food.

Results: Piglets were fed infant formula for the first three weeks after birth, with the fat content originating either from cow's milk or vegetable sources. The results showed that growth and brain development were similar regardless of fat type. The blood lipid profile varied significantly (as expected), but the composition of brain lipids remained relatively stable. Furthermore, we found that the immune system was positively influenced by fat from cow's milk. In a separate study, we established a piglet model with surgically induced cholestasis, meaning an absence of bile flow from the liver to the intestine—a complication seen in certain newborns. This condition led to liver changes and negative effects on the brain. The model enables future studies on nutritional and pharmaceutical interventions for patients with impaired fat digestion.

Conclusion: Overall, the results indicate that both vegetable and bovine milk fat are suitable for infant formula, regardless of birth weight. We observed no differences in brain development within the three-week study period. However, we cannot rule out that longer-term studies might reveal differences in brain development. The immune system was positively influenced by bovine fat, enhancing the ability to break down pathogens such as *E. coli*. This immunological capability is important, particularly for newborns who are vulnerable to infections. Finally, we conclude that the developed cholestasis model paves the way for new treatment options for infants with bile duct disorders.

7. Project aim

Dansk:

1. Vi ønskede at karakterisere udviklingen af fedtfordøjende enzymer i bugspytkirtlen ved anvendelse af grise som model for nyfødte børn. Vi ønsker at vise betydningen af alder ved fødsel, og hvordan mælkeernæring efter fødsel påvirker disse enzymer.
2. Vi ønskede at dokumentere effekter af mælkefedt og vegetabilsk fedt under forhold med nedsat galdeflow. Denne tilstand er velkendt, og medfører hospitalsindlæggelse
3. Vi ønskede at dokumentere tarm-, immunitet- og hjerneeffekter af bovint og vegetabilsk fedt, i en grise-model for lav- og normal fødselsvægt.

English:

1. We aimed to clarify the development of fat-digesting enzymes in the pancreas using pigs as a model for newborns. We want to demonstrate the impact of birth age and how milk nutrition after birth affects these enzymes.
2. We aimed to document the effects of milk fat and vegetable fat under conditions of reduced bile flow. This condition is well-known and often leads to hospitalization.
3. We aimed to document the effects of bovine and vegetable fat on the intestines, immunity, and brain in a pig model for low and normal birth weight.

8. Background for the project

Breast milk is widely recognized as the optimal source of nutrition for infants, providing a comprehensive array of essential nutrients and bioactive compounds necessary for healthy development. It not only supplies vital macronutrients but also supports the immune system and enhances cognitive function. However, in cases where breastfeeding is not possible or insufficient, infant formula is an alternative. One of the most important components of human milk is its fat content, which plays a fundamental role in energy provision and brain development.

Historically, bovine milk fat was the primary lipid source in infant formulas. However, due to economic and accessibility reasons, vegetable oils have largely replaced bovine milk fat in modern formulations. While vegetable oils offer a higher concentration of polyunsaturated fatty acids, they lack several essential lipids present in both human and bovine milk. These missing lipids include cholesterol, sphingomyelin, and short- and odd-chain fatty acids, all of which play key roles in infant growth and development. Further, one critical difference between lipid sources in breast milk and formula is the structural composition of milk fat globules. In human and bovine milk, fat is contained by the milk fat globule membrane (MFGM), a structure rich

in bioactive lipids and proteins. MFGM plays a significant role in emulsification, digestion and nutrient absorption, influencing metabolic processes in infants. Studies suggest that dairy-based lipids in infant formula can have beneficial effects on gut health and brain development. For example, bovine milk polar lipids have been found to enhance the hydrolysis and absorption of triacylglycerol (TAG) in neonatal piglets when compared to soy lecithin. Additionally, dairy fat blends enriched with α -linolenic acid have been associated with increased accretion of docosahexaenoic acid (DHA) in the brains of juvenile rats. Clinical studies further indicate that supplementing infant formula with MFGM can improve cognitive, language, and motor development in term-born infants. These benefits are particularly evident in infants born with intrauterine growth restriction (IUGR), a condition that can significantly affect early development.

IUGR infants face health challenges resulting from placental insufficiency, which can impair intestinal development and nutrient absorption. They are at an increased risk for neurodevelopmental impairments, including compromised white matter development, as well as postnatal infections. Animal studies, particularly those conducted on pigs with IUGR, have demonstrated that these developmental deficits can lead to learning difficulties and delayed cognitive function. Although research on dietary fat sources and their impact on neurodevelopment remains limited, ongoing investigations aim to determine whether different fat compositions in infant formula can influence lipid composition in the hippocampus and related neurodevelopmental outcomes.

Beyond neurodevelopment, early-life nutrition also plays a pivotal role in immune system development. Breast milk is known to promote the establishment of a healthy gut microbiota, enhance immune responses, and reduce the risk of postnatal infections. In this context, MFGM has been shown to inhibit leukocyte proliferation and cytokine production, indicating their potential role in immune regulation. Emerging research highlights the immunological benefits of supplementing infant formula with MFGM. Studies suggest that MFGM supplementation can modulate inflammatory responses by reducing interleukin production and decreasing the incidence of acute otitis media, a common infection in infants. Additionally, MFGM appears to protect the gut from inflammation induced by lipopolysaccharides, a major component of bacterial endotoxins. This protective effect may help in establishing a robust and resilient immune system during infancy, a period critical for long-term health outcomes.

Collectively, the lipid composition of infant formula is a critical factor influencing neurodevelopment and immune function. While modern formulas have made significant advancements in mimicking the composition of human milk, there remain key differences, particularly in lipid structure and bioactive components. Emerging evidence suggests that incorporating dairy-derived lipids, particularly those enriched with MFGM, could provide substantial benefits for both cognitive and immune system development. Further research is needed to refine infant formula formulations to better support the health and growth of infants, particularly those facing developmental challenges such as IUGR or neonatal cholestasis. With ongoing advancements in nutritional science, there is potential to develop more physiologically appropriate formulas that can bridge the gap between breast milk and formula feeding, ultimately improving health outcomes for all infants.

9. Sub-activities in the entire project period

WP1: Background: This WP was an expansion of an ongoing project within the NEOMUNE consortium financed by the Innovation Foundation Denmark. Relative to pigs born at term, we have described the influence of preterm birth on postnatal development of behavior, immunity, and intestinal function, but had no evidence of lipid digestive function (20,21). **Hypothesis:** We hypothesized that preterm neonatal piglets display reduced pancreatic lipase-activity, making them prone to fat-maldigestion. **Design and laboratory endpoints:** Pancreas samples from cesarean-derived piglets (term and preterm) were collected at days 1, 5, 11 or 26, to study ontogeny of lipase activity. After birth the pigs were provided parenteral nutrition support

for 5 days and minimal enteral nutrition with bovine colostrum. After day 5 they transitioned to full enteral nutrition with unpasteurized bovine milk. Lipase activity in collected pancreas samples was measured using established techniques at University of Lund.

WP2 Background: Bovine fat or vegetable fats were added to a base diet. To test fat fractions in a standardized manner, we designed a base diet consisting of whey protein isolate (DI-9224, 25 g/L final milk), casein (Miprodan 40, 25g/L final milk) and permeate (Variolac 855, 50g/L final milk). Bovine milk fat/cream was purchased and mixed into the base diet at 70 g/L milk. With their high contents of milk fat globule-membranes (MFGM) bovine fat dissolved readily in the base diet. For the vegetable fat group, we used soy lecithin as emulsifier. *Hypothesis:* Bovine fat, relative to vegetable fat, shows higher absorption and improves gut, immunity and brain function in neonatal pigs. *Design:* Pigs of either normal or low body weight were delivered at full term and fed experimental diets individually in cages for three weeks. *Laboratory endpoints:* Isolated leucocytes and CD8 cells (Th1/Th2/Th17) from blood samples were collected day 1 and day 10 and stimulated with TLR2/4 agonists (i.e. Staph. Epidermidis and LPS) to measure their cytokine production (IL-10, IL-1b, IL-8, IL-6, IFN, IL-17A). From the same set of samples an *ex vivo* neutrophil phagocytosis capacity test was performed. Plasma samples and collected tissue from hippocampus were used for quantitative lipidomics analysis at SDU to determine differences between the incorporation of fatty acids into distinct pools of membrane and storage lipids. Samples of the intestine were analyzed for their digestive enzyme activity, and mucosal dimensions were determined in H&E stained histological sections.

WP3. Background: Infants born with biliary atresia have reduced absorptive function in the gut and thus require milk replacers with maximum digestibility of the fat fraction. In WP3 we established a surgically induced cholestasis piglet model that mimics some of the symptoms of bile duct atresia. *Hypothesis:* The original hypothesis was that bovine milk fats, with high contents of MFGM constituents, would be superior to vegetable fat and increase the incorporation of lipid into brain, and improves immunity and gut functions in a piglet model of bile duct atresia. However, with the available monetary and personnel resources we had to make adjustments in hypothesis and design. We hypothesized that bile duct ligation would induce pathological changes in the liver and brain and thereby mimic the phenotype of bile duct atresia in human infants. *Design:* Neonatal piglets were subjected to either bile duct ligation or sham operation and fed milk replacer in individual cages for three weeks. Laboratory analysis is largely identical to WP 2.

10. Deviations

At the start of the project, we planned to include an experimental group that would be fed human milk fat mixed into a base diet. We began collecting frozen surplus breast milk from milk banks at local hospitals. However, we were concerned that freezing and subsequent thawing for fat isolation, followed by another freezing and thawing cycle, would degrade the quality of the fat too much. Since we did not expect to obtain reliable results, we decided to exclude it. Further, the challenging working conditions due to COVID, led to some delays, but we managed to complete most activities as planned. DDRF is acknowledged for providing financial support for a short extension of the PhD student who, due to COVID-19, was delayed in conducting the animal experiments. Finally, as mentioned in WP3 we had to redesign the *in vivo* part of the experiment to better fit with the economic and personnel resources. Thus, more emphasis was put on establishing and characterizing the bile duct ligation model. With this model at hand, we are now able to study intervention strategies.

11. Project results

The project led to 4 published manuscripts, which were in line with project descriptions from each of funding bodies (i.e. Novo Nordisk Foundation, Mille Food, The Research Council for Technology and Production). We provide in the following an overview of the key findings in each paper.

1.0 Henriksen NL, Asmussen KS, Pan X, Jiang PP, Mori Y, Christiansen LI, Sprenger RR, Ejasing CS, Pankratova S, Thyman T. Brain lipidomics and neurodevelopmental outcomes in intrauterine growth restricted piglets fed dairy or vegetable fat diets. *Sci Rep.* 2022 Feb 28;12(1):3303.

We investigated the effects of dietary fat sources on neurodevelopment, focusing on intrauterine growth-restricted (IUGR) piglets as a model for low-birth-weight infants. The study compared the impacts of bovine milk fat (MILK) and vegetable oil (VEG) diets on lipid composition, brain development, and neurobehavioral outcomes. Neonatal piglets of either normal body weight (NBW) or low body weight (IUGR) were purchased from a local supplier, and transported to the experimental facility where they were single-housed for three weeks. The two experimental diets were fed in meals every 3 hours using a 2x2 factorial design. There were no clinical complications in any group, and increments in body weight was similar across VEG and MILK. The graph to the right indicates NBW and IUGR pooled across diet type, and VEG and MILK pooled across body weight.

The key findings from the lipidomics assay showed the plasma lipid profiles were significantly influenced by the dietary fat source, with 82% of lipid molecules showing differences. Further, in the hippocampus, 11% of lipid molecules differed between diet groups, with MILK diets upregulating most of these lipids, particularly glycerophospholipids. Interestingly, the major differences in lipid species found in plasma between VEG and MILK (Figure 2, right upper panel) were not associated with similar differences in the hippocampus. In fact, the number of down- and up-regulated lipid species was much smaller, yet with the majority of differential lipid species tilted toward upregulation in the MILK. While we can only speculate, the inference of this observation may be that the brain has selective capacity or de novo capacity toward lipid species that are represented in bovine milk.

IUGR piglets exhibited lower absolute brain weights, reduced grey and white matter volumes, and impaired motor function and behavior compared to normal birth weight (NBW) piglets (Figure 3). Diet had minimal influence on these structural deficits or functional impairments. Likewise, cognitive performance and cere-

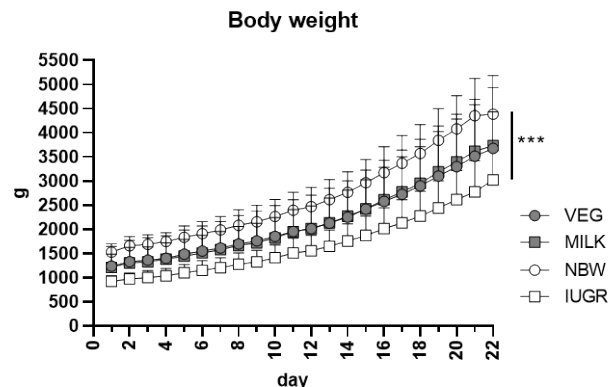


Figure 1. Body weight increments in pigs of either normal birth weight (NBW) or low birth weight (IUGR). Both groups were fed milk replacers with the fat fraction derived from either vegetable (VEG) or bovine milk (MILK) origin. All groups n=18

bellar gene expression profiles were similar across dietary groups and between IUGR and NBW piglets. Interactive effects between diet and birth weight were minor. While MILK diets altered plasma and hippo-

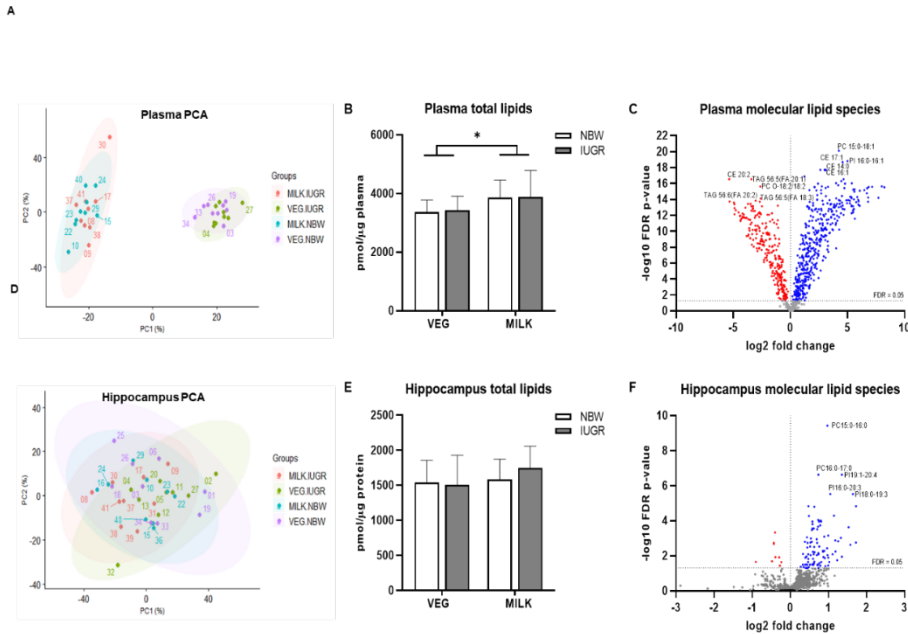


Figure 2. Principal component analysis (PCA) (left panels), plasma and hippocampus total lipids (middle panels) and volcano plots of plasma and hippocampus lipid species (right panels). In volcano plots, red indicate down regulated lipid species, whereas blue indicate upregulated lipid species in MILK relative to VEG

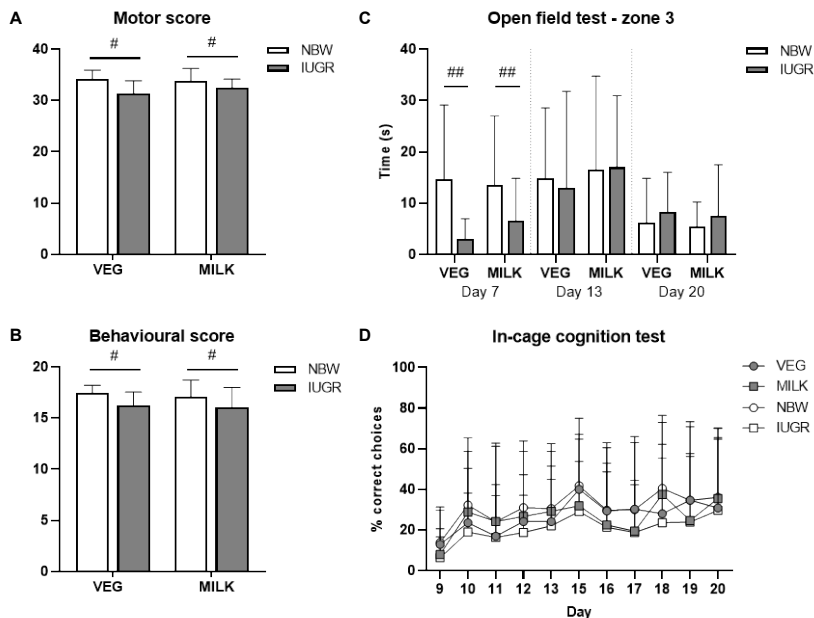


Figure 3. Motor score, behavioral score, open field test and in-cage cognition test in pigs of either normal birth weight (NBW) or low birth weight (IUGR). Both groups were fed milk replacers with the fat fraction derived from either vegetable (VEG) or bovine milk (MILK) origin. All groups n=18

campal lipidomes more than VEG diets, they did not mitigate the neurodevelopmental impairments caused by IUGR.

We conclude that while the source of dietary fat significantly affects lipid profiles in plasma and hippocampus, it has limited impact on improving structural or functional brain deficits in IUGR piglets. This highlights the complexity of addressing neurodevelopmental challenges in low-birth-weight infants through dietary interventions.

2.0 Bæk O, Skadborg K, Muk T, Amdi C, Heegaard PMH, Thymann T, Nguyen DN. Infant Formula Based on Milk Fat Affects Immune Development in Both Normal Birthweight and Fetal Growth Restricted Neonatal Piglets. *Nutrients*. 2021 Sep 22;13(10):3310.

This study was based on the same experiment as presented above. We investigated the effects of infant formula based on milk fat (MILK) versus vegetable oil (VEG) on immune development in normal birthweight (NBW) and intrauterine growth restricted (IUGR) neonatal piglets. Various immune parameters were evaluated, including blood cell counts, T cell populations, neutrophil function, and gene expression after bacterial stimulation. We found that Milk-fat feeding decreased blood neutrophil counts and improved neutrophil function compared to vegetable oil-based formula. Milk-fat formula transiently reduced leucocytes' expression of genes related to adaptive and innate immunity as well as energy metabolism after in vitro stimulation with *Staphylococcus epidermidis*. We found only a few interactions between milk-fat type and birthweight status, suggesting the effects were similar for both NBW and IUGR piglets. Further, piglets fed milk-fat-based formula showed improved neutrophil maturation and suppressed pro-inflammatory responses compared to those fed vegetable-oil-based formula.

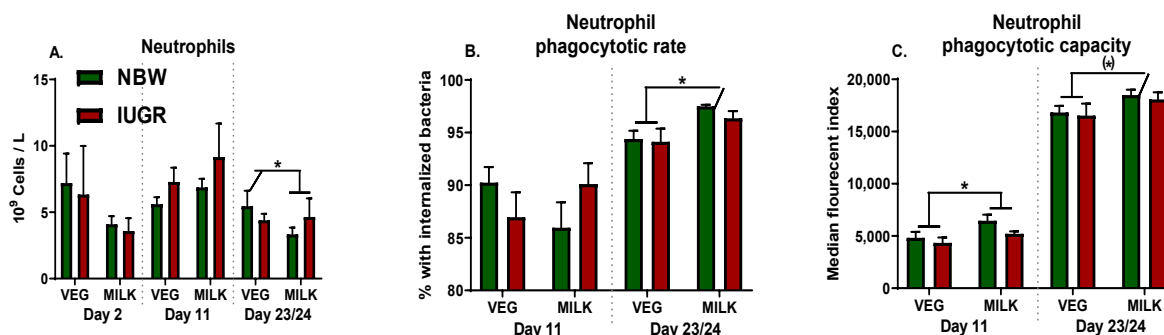


Figure 4. Ex vivo neutrophil phagocytosis test using fluorescently labeled *E. Coli*. Blood samples were collected day 11 and day 23/24 from IUGR and NEW pigs fed either VEG or MILK experimental diets. n=9 for all subgroups.

We conclude that replacing vegetable oil with bovine milk fat in infant formula may improve immune development in both NBW and IUGR neonates, particularly by enhancing neutrophil function and modulating inflammatory responses

3.0 Henriksen NL, Hansen SH, Lycas MD, Pan X, Eriksen T, Johansen LS, Sprenger RR, Ejsing CS, Burrin DG, Skovgaard K, Christensen VB, Thymann T, Pankratova S. Cholestasis alters brain lipid and bile acid composition and compromises motor function in neonatal piglets. *Physiol Rep*. 2022 Jul;10(13):e15368.

We here studied how neonatal cholestasis affects brain lipid and bile acid composition and motor function in piglets. We developed a piglet model of neonatal cholestasis using bile duct ligation surgery. The surgical procedures were initially established and are presented in a separate paper outside the current project. Using this model, we found that bile duct-ligated (BDL) piglets showed sensory-motor deficits compared to sham-operated controls. The brain bile acid profile in BDL piglets shifted towards more hydrophilic and conjugated bile acids, and lipidomic analysis of the cerebellum revealed a decrease in total lipids, including phosphatidylinositols and phosphatidylserines, and an increase in lysophospholipid species. Further, gene expression analysis of the cerebellum showed elevated expression of genes related to inflammation and tissue damage in BDL piglets.

The study suggests that alterations in brain bile acids and lipids may contribute to motor deficits observed in neonatal cholestasis. This research provides new insights into how neonatal cholestasis affects the developing brain at a molecular level and its potential relationship to motor function impairments. The findings may help explain the neurodevelopmental deficits observed in infants with neonatal cholestasis. The inference of our findings are that therapeutic approach should aim at reducing plasma bile acids levels (possibly via increased kidney clearance) to protect the brain from toxic levels of bile acids.

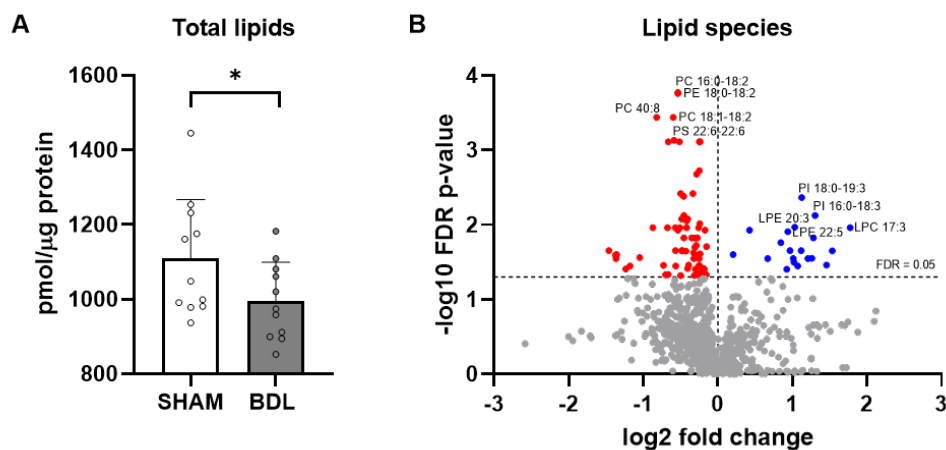


Figure 5. Total lipids (left panel) and volcano plot of downregulated (red) and upregulated (blue) lipid species in BDL relative to SHAM operated piglets. The samples were derived from the cerebellum, n=8-11.

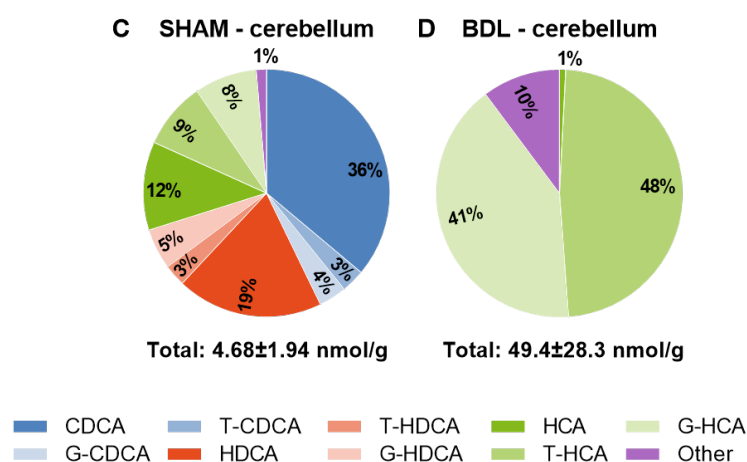


Figure 6. Bile acid profile in the cerebellum of pigs exposed of BDL or SHAM, n=8-11.

4.0 Arévalo Sureda E, Pierzynowska K, Weström B, Sangild PT, Thymann T. Exocrine Pancreatic Maturation in Pre-term and Term Piglets Supplemented with Bovine Colostrum. Front Nutr. 2021 Jun 24;8:687056.

We investigated the development of the exocrine pancreas in preterm and term piglets as a model for human infants. We explored how preterm birth and bovine colostrum supplementation affected pancreatic enzyme content (trypsin, amylase, and lipase). We hypothesized that pancreatic enzyme content in piglets is immature following preterm birth but can be stimulated by early colostrum supplementation. Piglets were delivered by cesarean section either preterm or term. The levels of trypsin, amylase, lipase, and total protein were measured in pancreatic tissue samples. Three experiments were conducted, which included comparisons of preterm and term piglets at different ages, and different feeding regimens with either bovine colostrum or total parenteral nutrition (TPN). The results showed that preterm piglets had lower levels of pancreatic trypsin, amylase, and total protein compared to term piglets. Colostrum feeding led to decreased trypsin and increased amylase contents expressed per gram of tissue. Lipase activity decreased with advancing gestational age at birth and postnatal age, showing no consistent response to colostrum feeding. In conclusion, amylase and trypsin are immature following preterm birth, but rapid postnatal increases occur with colostrum intake, demonstrating an adaptation capacity. Lipase is less affected by age and feeding. The findings suggest that dietary interventions like colostrum supplementation can influence the development and maturation of the exocrine pancreas in preterm infants.

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

Based on the positive effects observed on the immune system, it would be relevant to document whether the same beneficial effects can be achieved with a combination of milk fat and plant-based fat. The results of the present project contribute to the value assessment of the fat fraction from cow's milk. The project is

designed to investigate the effect of intact cream, which implies that the observed effects may potentially originate from subcomponents of the cream (triglycerides, MFGM). It would be valuable for the dairy industry to clarify whether the beneficial effects of cream can be reproduced by focusing on isolated fractions of cream, or whether they are only achieved when the cream remains intact. In addition to applications in human milk replacers, the MFGM fraction of cream in particular is expected to have positive effects in piglet production.

If the project contributes to improved milk replacers, the direct societal benefits will likely include reduced morbidity among children and piglets fed milk replacers. However, potential positive effects in children would require documentation through clinical studies. Indirect societal effects through increased value of cream cannot be ruled out, but are difficult to assess realistically.

The establishment of the cholestasis model may lead to the testing of various nutritional and pharmacological treatment approaches. If it proves possible to improve the clinical condition of this specific patient group (approximately 20 children per year in Denmark), this could result in fewer sick days and reduced hospital costs.

13. Communication and knowledge sharing about the project

Papers in international journals:

Henriksen NL, Asmussen KS, Pan X, Jiang PP, Mori Y, Christiansen LI, Sprenger RR, Ejsing CS, Pankratova S, Thymann T. Brain lipidomics and neurodevelopmental outcomes in intrauterine growth restricted piglets fed dairy or vegetable fat diets. *Sci Rep.* 2022 Feb 28;12(1):3303.

Bæk O, Skadborg K, Muk T, Amdi C, Heegaard PMH, Thymann T, Nguyen DN. Infant Formula Based on Milk Fat Affects Immune Development in Both Normal Birthweight and Fetal Growth Restricted Neonatal Piglets. *Nutrients.* 2021 Sep 22;13(10):3310.

Henriksen NL, Hansen SH, Lycas MD, Pan X, Eriksen T, Johansen LS, Sprenger RR, Ejsing CS, Burrin DG, Skovgaard K, Christensen VB, Thymann T, Pankratova S. Cholestasis alters brain lipid and bile acid composition and compromises motor function in neonatal piglets. *Physiol Rep.* 2022 Jul;10(13):e15368.

Arévalo Sureda E, Pierzynowska K, Weström B, Sangild PT, Thymann T. Exocrine Pancreatic Maturation in Pre-term and Term Piglets Supplemented with Bovine Colostrum. *Front Nutr.* 2021 Jun 24;8:687056.

Oral presentationst scientific conferences, symposiums etc.:

We have conducted a full-day seminar on nutrition and dietary fat at the University of Copenhagen in September 2021. We have presented the results on plant-based versus bovine fat at EuroTier in Hannover in 2023 and at Nukamel in Antwerp in 2024. In addition, we presented the cholestasis model at an internal meeting at Rigshospitalet.

14. Contribution to master and PhD education

We have hosted an Erasmus student, Esther Sureda, who, with her expertise in enzyme analysis, has carried out analyses of pancreatic enzymes. Furthermore, the project has formed the basis for the majority of a PhD education undertaken by Nicole Henriksen.