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Permeat som ernæringstilskud efter moderat fejlernæring

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1. Title of the project

Permeat som ernæringstilskud efter moderat fejlernæring

Permeate as nutritional supplement after moderate malnutrition

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4. Sources of funding

Danish Dairy Research Foundation and University of Copenhagen

5. Project period

Project period with DDRF funding:	January 2015- July 2016
Revised:	September 2015- July 2017

6. Project summary (English)

Background. Malnutrition includes stunted growth, wasting and disturbed electrolyte levels in the blood. The small intestine becomes less functional and patients may present symptoms like diarrhea. Patients with moderate malnutrition, which accounts for most cases relative to severe malnutrition, may display some of these symptoms although not as life threatening as severe cases. Refeeding of patients with moderate malnutrition is largely based on corn-soy blends with added sucrose. It is however unclear if sugars of dairy origin may be better suited for infants with regards to growth, clinical and paraclinical endpoints and endpoints related to gut function and the gut microbiota. The dairy product 'whey permeate' consists mainly of lactose and milk minerals, and may be a good nutritional source to stabilize gut function and circulate electrolytes during refeeding.

Methods. Four-week old pigs were fed a pure maize diet for 17 days to induce symptoms of moderate malnutrition. At this stage tissues were collected from a subsample of pigs to document the degree of malnutrition (MAL, n=7). The remaining pigs were fed a corn-soy blend until day 42, with added sucrose (SUC, n=11), lactose (LAC, n=11) or whey permeate (PERM, n=11), i.e. a dairy product high in lactose and milk minerals. All three products were added at 10% of the total diet as fed.

Results. Malnutrition induced slow growth and resulted in decreases in phosphate, albumin and bilirubin, while sodium, potassium and markers of liver dysfunction were increased. During the following re-feeding period, most physiological endpoints became normalized (e.g. growth, body composition, organ weights, markers of muscle and liver function), regardless of whether the diets were enriched with sucrose, lactose or permeate.

Interestingly, pigs refed with PERM showed a lowering of electrolyte, protein and blood cell concentrations relative to SUC and LAC. This indicates an expansion of plasma volume in PERM, resulting in a general dilution of these blood markers. Further, PERM increased urine creatinine concentration versus LAC and SUC, which indicates lower urine production in PERM. Collectively, the results suggest that PERM, via its content of milk minerals, can retain and accumulate fluid into the blood vessels.

We also studied the effect of the SUC, LAC and PERM on the intestinal function and the microbiome of the upper and lower parts of the gastrointestinal tract. In brief we found that the activity of important digestive enzymes was largely similar for MAL, LAC, PERM and SUC, suggesting that the digestive function at this stage of life and at this level of moderate malnutrition, was not sensitive to the experimental diets. Likewise, the gut microbiome was largely similar for all groups, with only marginal differences between SUC, LAC and PERM.

Conclusion. Refeeding after moderate malnutrition with a corn-soy based diet with added permeate, expands the plasma volume and reduces urine production relative to diets enriched with sucrose or lactose. Further studies are required to determine where the fluid for plasma volume expansion is derived from, as this is important for the interpretation of the effects of permeate. However, for patients with low blood pressure but normal heart and kidney function, expansion of plasma volume can be regarded as a benefit to support perfusion in peripheral tissues.

6. Project summary (Danish)

Baggrund. Underernæring er bl.a. forbundet med nedsat vækst og forstyrret elektrolytniveau i blodet. Tyndtarmen bliver mindre funktionel, og patienter kan udvise symptomer som nedsat fordøjelse og diarré. For patienter som lider af moderat underernæring, hvilket antalsmæssigt er flere end dem, der er svært underernærede, ses lignende symptomer, omend i mildere grad. Reernæring af patienter med moderat underernæring er hovedsagelig baseret på majs-sojablandinger med tilsat rørsukker. Det er imidlertid uklart, om andre sukkerarter som mælkesukker (laktose) eller en kombination af laktose og mælkemineraler, kan have gavnlige effekter på kliniske og fysiologiske målinger af vigtige organer som tarm, kredsløb og immunsystem.

Metoder. Fire uger gamle grise blev fodret udelukkende med majs-pulver i 17 dage for at fremkalde symptomer på moderat underernæring. På dette stadie blev der opsamlet væv fra syv grise for at dokumentere graden af underernæring (MAL, n = 7). De tilbageværende grise blev fodret med en majs-sojablanding indtil dag 42, med tilsat rørsukker (=sukrose) (SUC, n = 11), laktose (LAC, n = 11) eller valle-permeat (PERM, n = 11). Alle tre produkter blev tilsat som 10% af den samlede diæt.

Resultater. Den indledende periode med underernæring, gav reduceret vækst og fald i fosfat, albumin og bilirubin, mens natrium, kalium og markører for leverdysfunktion blev forøget. Dette bekræfter, at grisene var underernærede. Når grisene derefter blev re-ernæret, blev de fleste fysiologiske parametre normaliseret (vækst, kropssammensætning, organvægte, markører for muskel- og leverfunktion m.fl.), uagtet hvilken re-ernæringsdiæt, der blev givet. Det viste sig endvidere, at grise i PERM-gruppen havde en markant reduktion elektrolyt-, protein- og blodcellekoncentrationer i forhold til SUC og LAC. Dette indikerer en udvidelse af plasmavolumen i PERM, hvilket medfører generel fortynding af disse blodmarkører. Endvidere havde PERM forøget kreatinin-koncentration i urinen i forhold til LAC og SUC, hvilket indikerer lavere urinproduktion i PERM. Samlet set tyder resultaterne på, at PERM, via dets indhold af mælkemineraler, kan tilbageholde og akkumulere væske i blodkarrene, så blodvolumen øges.

Vi undersøgte også effekten af SUC, LAC og PERM på tarmfunktionen og mikrobiomet i de øvre og nedre dele af mave-tarm-kanalen. Resultatet var, at aktiviteten af vigtige fordøjelsesenzymer stort set var ens for alle forsøgsgrupper, hvilket tyder på, at fordøjelsesfunktionen på dette stadie af livet og ved denne grad af underernæring, ikke var følsom over for de eksperimentelle diæter. Tilsvarende fandtes kun marginale forskelle i tarmens bakteriesammensætning mellem SUC, LAC og PERM.

Konklusion. Når der efter moderat underernæring, re-ernæres med en majs-sojabaseret diæt med tilsat permeat, udvides plasmavolumenet og urinproduktionen reduceres i forhold til diæter med tilsat rørsukker eller laktose. Yderligere undersøgelser er nødvendige for at afgøre, hvor væsken til plasmavolumen-udvidelsen kommer fra, da dette er vigtigt for fortolkningen af virkningerne af permeat. For patienter med lavt blodtryk men normal hjerte- og nyrefunktion, kan ekspansion af plasmavolumen betragtes som en fordel for at understøtte gennemstrømning i perifere væv.

7. Project aim

The project aims were to:

1. To verify recovery from electrolyte fluctuations in response to refeeding with permeate.
2. To test intestinal function and integrity in response to refeeding with permeate.
3. To study growth velocity and body composition in response to refeeding with permeate.
4. To determine the effects of lactose and minerals from permeate separately and together.

8. Background for the project

In developing countries, children less than 5 years of age are highly sensitive to malnutrition with 178 million children showing stunted growth, 55 million showing wasting, and 3.6 million that die from malnutrition every year (Black et al., 2013). The pathogenesis of starvation or malnutrition is complex as it affects all organ systems simultaneously. The intestinal surface becomes smaller and permeable, and the gut microbiota changes giving rise to symptoms like diarrhea. Other pathological changes include fatty liver, retention of bile, jaundice, and compromised function of the heart, kidney, adrenal glands and pituitary gland. Paradoxically, refeeding of severely malnourished patients can lead to clinical complications like decreased concentration of phosphate and magnesium in the blood as well as disturbed fluid balance. These acute pathological changes relate to the increased energy intake and increases in mitochondrial activity and cellular influx of electrolytes, particularly phosphate. From this notion, diets with adequate amounts of bioavailable electrolytes including phosphate, potassium and magnesium are required to prevent refeeding syndrome and to stabilize fluid balance and circulatory function.

The dairy product 'permeate' is a derivative of whey, where most protein has been removed. The product therefore largely represents a mixture of lactose (approximately 85% of the product) and milk minerals. We have previously shown in a malnourished piglet model that addition of permeate to a corn/soy-based diet secures plasma phosphate during refeeding relative to a control group without permeate (Hother et al., 2017), suggesting that the mineral fraction, or the mineral fraction in combination with lactose helps stabilize electrolyte levels during refeeding.

Severe malnutrition may induce changes of the structure in the intestinal surface, resulting in a degree of lactase deficiency. Starter therapeutic diets like F-75 are therefore designed to have low lactose content, in particular for patients with persistent diarrhea. In contrast, for patients with sufficient lactase activity, it remains unclear whether regeneration of the intestine is better stimulated with lactose relative to sugars like sucrose which are normally used in rehabilitation diets. A previous study has shown that the derivatives of the lactose molecule, i.e. glucose and galactose appear in the portal vein in a very different ratio (i.e. lower galactose level) relative to their 1:1 ratio in the lactose molecule (Thymann et al., 2009). This indicates that a large part of the galactose may be consumed during the first passage through the intestinal wall. Whether there is a preference for galactose as a substrate is however not understood in detail.

On this background, we hypothesized that: 1) relative to sucrose, lactose improves gut function and integrity during refeeding of moderately malnourished patients, and 2) the combined effect of lactose and milk minerals in the permeate product, supports clinical and physiological indices of recovery from moderate malnutrition.

The following experimental diets were used:

Table 1. Dietary composition of malnutrition-induction diet (MAL) and experimental feeding diets LAC, PERM and SUC[1].

	MAL	LAC	PERM	SUC
Ingredients (g/kg (as fed))				
Maize	1000	780	780	780
Whole soybean	-	200	200	200
Mineral and vitamin mix[2]	-	2.0	2.0	2.0
Monocalcium phosphate		8.0	8.0	8.0
Potassium chloride		7.6	7.6	7.6
Lactose[4]	-	100	-	-
Permeate[4]	-	-	100	-
Sucrose	-	-	-	100
Nutrient composition				
Energy (MJ/kg)	9.4	16	16	17
Crude protein (Nx6.25) (g/kg)	90	150	130	170
Crude Fat (g/kg)	43	62	57	91
Fiber (g/kg)	NA	25	23	20
Macro minerals (g/kg):				
Calcium	0.08	5.6	4.8	6.3
Phosphorus				
Total	4.5	4.8	4.8	5.2
Phytic acid	4.5	3.1	2.9	2.5
Non-phytic acid (% of total)	0(0)	1.7(36)	1.9 (39)	2.7 (51)
Mineral ratios				
Ca:P	0.02	1.2	1.0	1.2
Nutrient density (mg/1000 kcal)				
Phosphorus[6]	-	450	500	670

1CSB+ was kindly donated by Michiels Fabrieken NV, (Michiels Fabrieken NV, Zulte, Belgium) according to World Food Programme (WFP) guidelines. The MAL diet, i.e. pure maize was produced and kindly donated by DLG, Gråsten, Denmark. 2Vitamin/Mineral FBF-V-10 provided the following (units/kg diet when added 2 g/kg): vitamin A, 16,640 IU; thiamine, 1.28 mg; riboflavin, 4.48 mg; niacin, 48 mg; pantothenic acid, 67 mg; vitamin B6, 17 mg; folate, 600 µg, vitamin B12, 20 µg; vitamin C, 1 g; vitamin D, 40 µg; vitamin E, 83 µg; vitamin K, 1 mg; iron, 65 mg; zinc, 50 mg; and iodine, 400 µg. 3Monocalcium phosphate (monohydrate), potassium chloride and calcium carbonate provided nutrients (units/kg diet) were added as follows: potassium, 4 g; calcium, 5 g; and phosphorus, 2 g. 4Variolac®855 (Arla Foods Ingredients, Viby, Denmark) (composition: 87% lactose, 2.2% protein, Total mineral 6%). 5Premium Lactose (Arla Foods Ingredients, Viby, Denmark) Composition: 99.7% lactose. Dansukker 5Energy, protein, fat and fiber analyzed by SGS (SGS, Antwerp, Belgium).

9. Sub-activities in the entire project period

The start of the project was postponed from January 2015 to September 2015. Before we launched the animal experimentation, an attempt was made to develop an *ex vivo* system to video-record and quantify intestinal peristaltic movements. Although the assay would have provided potential important data on gut functionality, we could not produce reliable data, due to anesthesia-induced paralysis of the intestinal tissue. We therefore focused our attention to other key endpoints, and expanded the panel of measurements to also include the gut microbiome.

The animal experimentation work was carried out September-October 2016, and tissue and blood analysis followed. A draft full manuscript has been produced and an abstract has been published in the proceedings for ESPGHAN (European Society for Pediatrics, Gastroenterology, Hepatology and Nutrition), Geneva, May 2018. The abstract was selected for oral presentation at the conference.

10. Project results

Induction of malnutrition: Despite the suboptimal nutritional composition of pure maize, there was a small increase in body weight during the malnutrition induction phase. Linear body growth, as indicated by crown-to-rump length, did not appear to be as sensitive to malnutrition as body weight (Fig. 1). Likewise, the daily clinical assessments of 1) response to stimuli, 2) posture and gait, 3) skin turgor and 4) diarrhea, were not affected (data not shown). Although these clinical parameters were unaffected, voluntary feed consumption of the pure maize did indicate some loss of appetite, with a decrease from approx. 300 g/day on day 1 to approx. 150g/d at the end of the malnutrition period. As expected, there were no differences in feed consumption between any groups at this stage, confirming a proper randomization at the beginning of the experiment (Fig. 1).

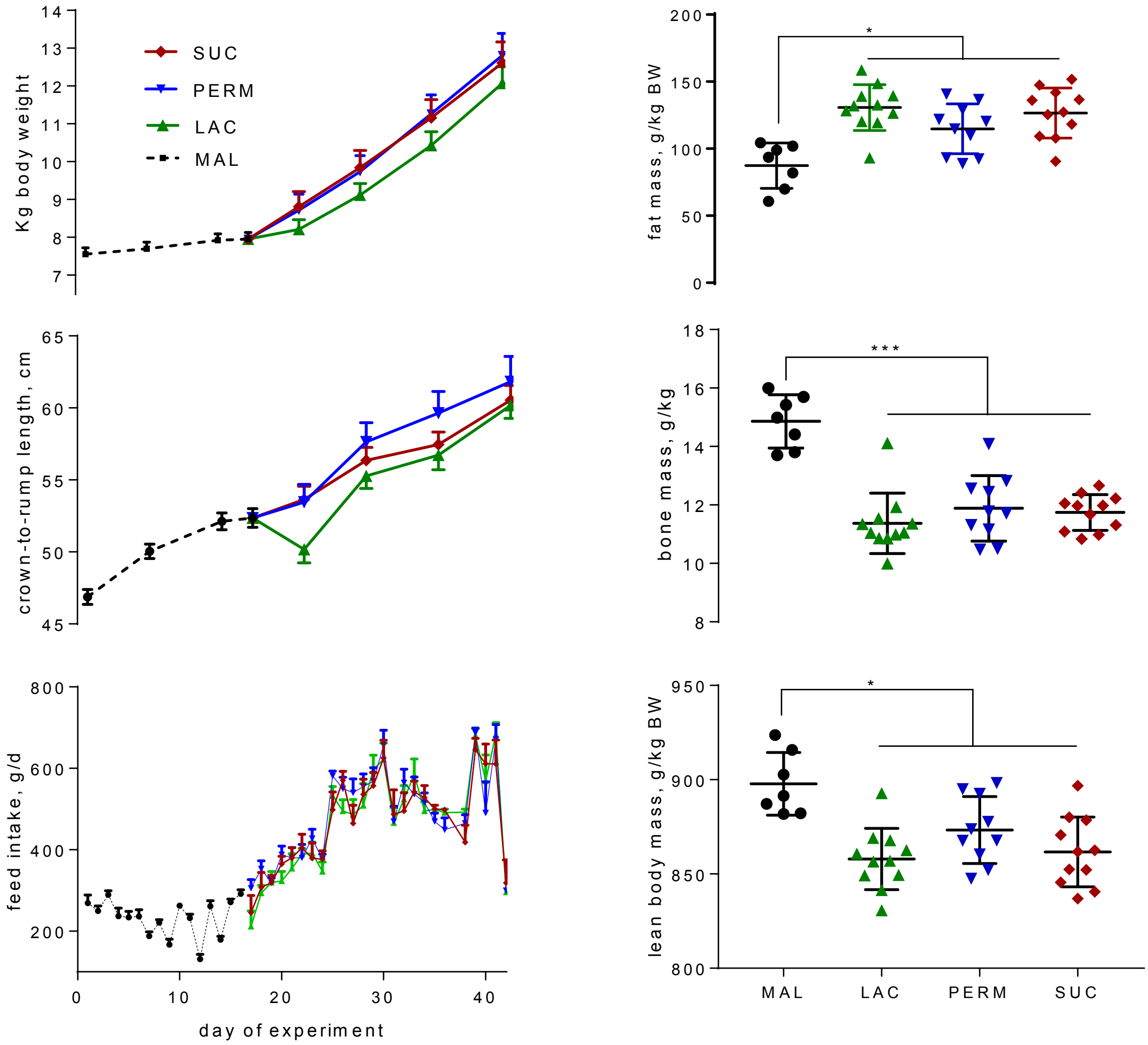

Figure 1. Growth, body composition and feed intake in pigs fed a nutritionally suboptimal pure maize diet (MAL, n=7), or refed with corn-soy blends with added lactose (LAC, n=11), permeate (PERM, n=11) or sucrose (SUC, n=11). Panels indicate absolute body weight (A), crown-to-rump length (B), voluntary feed intake (C), relative fat mass (D), relative bone mass (E), and relative lean body mass (F). All data are expressed as means ± std. err.

As further indicators of malnutrition, blood cell markers in the malnourished pigs (MAL) versus the newly weaned (i.e. before malnutrition, WEAN) showed decreases in hematocrit, hemoglobin concentration (p=0.06), mean cell volume and blood platelet count, while red blood cell count was not affected (Fig. 2). Interestingly the stimulation of whole blood with a TLR2 or TLR4 agonist (i.e. to mimic acute cellular immune responses to infections with gram-positive and gram-negative bacteria), showed a clear reduction in the ability to secrete TNF-α (a proinflammatory compound) following stimulation with either agonist. For IL-10 8an anti-inflammatory compound) the data were associated with too high variation to identify any difference between WEAN and MAL (see Appendix, Fig. 5).

Moreover, the concentration of circulatory electrolytes showed decreases in phosphate and iron, while sodium and potassium were increased. This was further associated with lowering of glucose, albumin, bilirubin, aspartate transaminase and creatine kinase, while alanine aminotransferase and gamma-glutamyl transferase were increased. Calcium, magnesium, urea and creatinine did not change during the induction of malnutrition (Fig. 3).

Collectively, the data indicate clear signs of moderate malnutrition, including reduced appetite and growth and reduced liver function and altered profile of blood cells and electrolytes.

Refeeding: Following introduction of the three refeeding diets, there was a rapid increase in voluntary feed intake and growth velocity for all groups (Fig. 1). Increment in body weight during refeeding was similar between permeate (PERM), lactose (LAC) and sucrose (SUC). All three diets were well tolerated and voluntary feed consumption was similar across the groups (Fig. 1). For body composition there was a clear increase in relative fat mass and a decrease in relative bone mass for the three refeeding groups relative to MAL, whereas relative lean mass did not change. Among the three refeeding groups there were no differences in body composition (Fig. 1). The general difference between MAL and the three refeeding groups was further associated with increases in the relative weight of the small intestine, large intestine, liver and adrenal glands, while the relative weight of the kidney and heart decreased. The organ weights were generally similar among the three refeeding groups, except for the spleen weight, which was higher in PERM versus LAC (Table 2).

Table 2. Organ weights relative to body weight in malnourished pigs (MAL) and in pigs refed with corn-soy blends with either added sucrose (SUC), permeate (PERM) or lactose (LAC). Data represent means ± sd, and different superscript letter within a row indicate significant difference.

	MAL	SUC	PERM	LAC
n	7	11	11	11
Stomach, g/kg	9.44 ± 1.24	9.74 ± 1.61	9.45 ± 1.03	9.64 ± 0.72
Small intestine, g/kg	37.7 ± 5.8^{a}	44.9 ± 4.6^{ab}	45.1 ± 6.1^{ab}	48.0 ± 6.9^{b}
Colon, g/kg	12.7 ± 2.7^{a}	24.3 ± 4.0^{b}	21.1 ± 2.7^{b}	23.4 ± 5.7^{b}
Liver, g/kg	27.1 ± 4.4	30.6 ± 2.7	33.7 ± 4.4	32.1 ± 3.2
Spleen, g/kg	5.81 ± 1.25^{ab}	5.57 ± 1.18^{ab}	6.45 ± 1.27^{a}	4.37 ± 1.52^{b}
Kidney, g/kg	5.65 ± 0.93^{a}	4.77 ± 0.37^{b}	4.56 ± 0.60^{b}	4.77 ± 0.61^{b}
Adrenal, g/kg	0.09 ± 0.06^{a}	0.13 ± 0.02^{b}	0.12 ± 0.02^{ab}	0.12 ± 0.02^{ab}
Heart, g/kg	5.21 ± 0.83^{a}	4.25 ± 0.36^{b}	4.54 ± 0.41^{ab}	4.18 ± 0.59^{b}
Lungs, g/kg	12.1 ± 1.4	10.7 ± 1.3	11.3 ± 3.2	11.9 ± 3.8

The hematologic profile showed a marked increase in the number of thrombocytes in the three refeeding groups relative to MAL, whereas total leucocyte count was unaffected (Fig. 2) Mean corpuscular hemoglobin concentration (calculated as hemoglobin concentration divided by hematocrit), showed a general decrease in the three refeeding groups relative to MAL, which was mainly due to an increase in hematocrit in the three refeeding groups. Whereas this ratio and mean cell volume (calculated as hematocrit divided by erythrocyte number) were similar among the three refeeding groups, there was a marked lowering of both erythrocyte number, hematocrit and hemoglobin concentration in PERM versus both LAC and SUC. There were no major changes in white blood cell numbers, except a small increase in basophil count and decrease in eosinophil count in the three refeeding groups relative to MAL (Fig. 2).

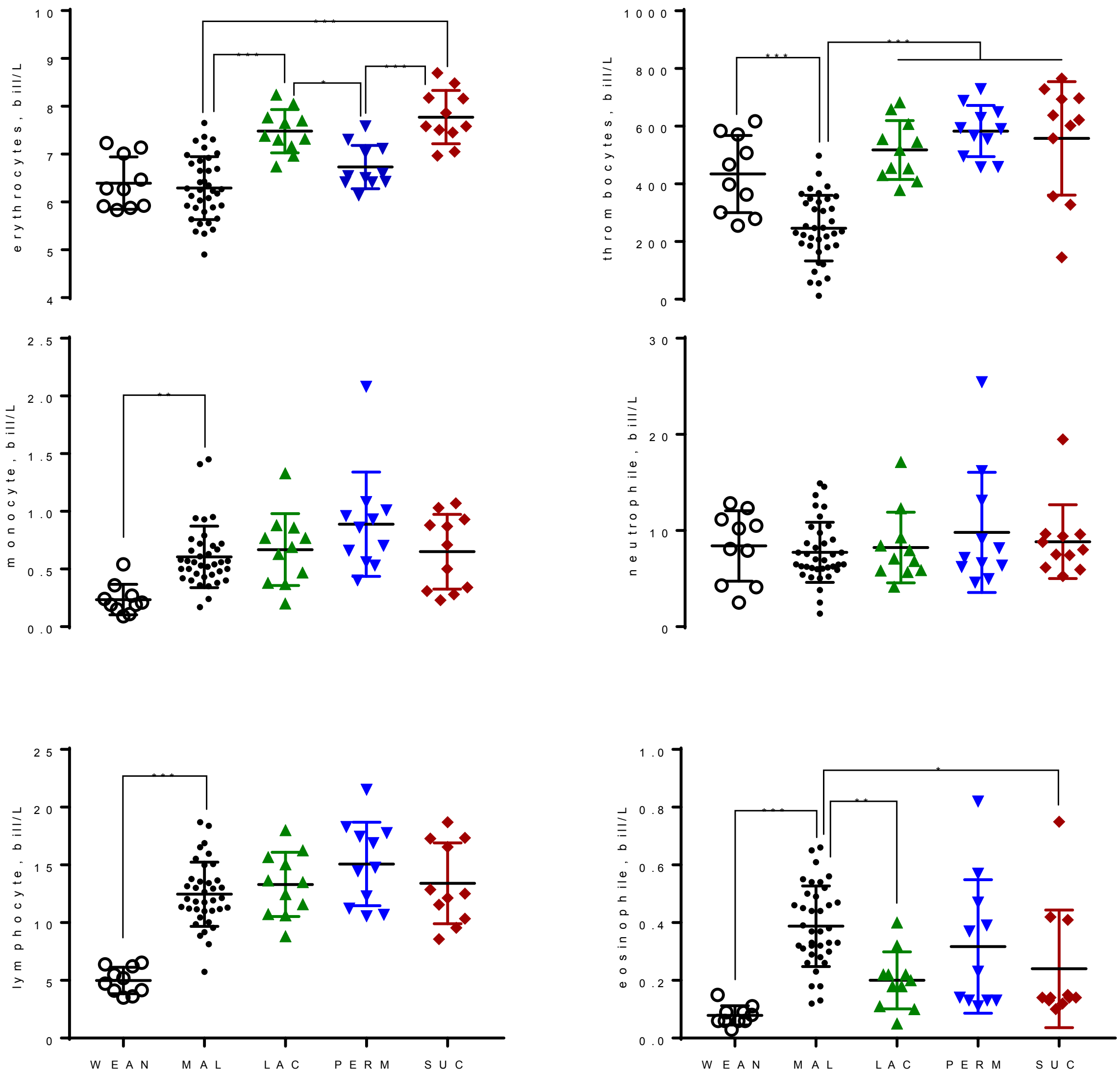

Figure 2. Hematological profile of newly weaned pigs (WEAN, n=10), and pigs fed a nutritionally suboptimal pure maize diet (MAL, n=40), or refed with corn-soy blends with added lactose (LAC, n=11), permeate (PERM, n=11) or sucrose (SUC, n=11). Panels indicate the number of erythrocytes (A), thrombocytes (B), monocytes (C), neutrophils (D), lymphocytes (E), and eosinophiles (F). All data are expressed as means ± std. err.

Interestingly, the stimulation of whole blood with a TLR2 agonist, showed a general increase in the secretion of TNF-α for all three refeeding groups relative to MAL, which may indicate recovery of the ability to mobilize a proinflammatory response (see appendix, Fig. 5).

Refeeding was associated with a marked reduction in the enzymes: creatine kinase, alkaline phosphatase, alanine aminotransferase, aspartate transaminase, bilirubin and creatinine in the three refeeding groups relative to MAL, whereas albumin was only increased in SUC and LAC (Fig. 3). Refeeding was also generally associated with an increase relative to MAL in plasma phosphate, magnesium and potassium. Interestingly, there was a marked lowering of calcium, magnesium and iron, whereas there was an increase in sodium in

PERM versus LAC and SUC. Although these differences may be a direct effect of the different mineral content of PERM versus LAC and SUC, the lowering in PERM of both, calcium, magnesium, iron, albumin, hematocrit, erythrocytes and hemoglobin, presumably reflects an expansion of plasma volume in PERM versus LAC and SUC.

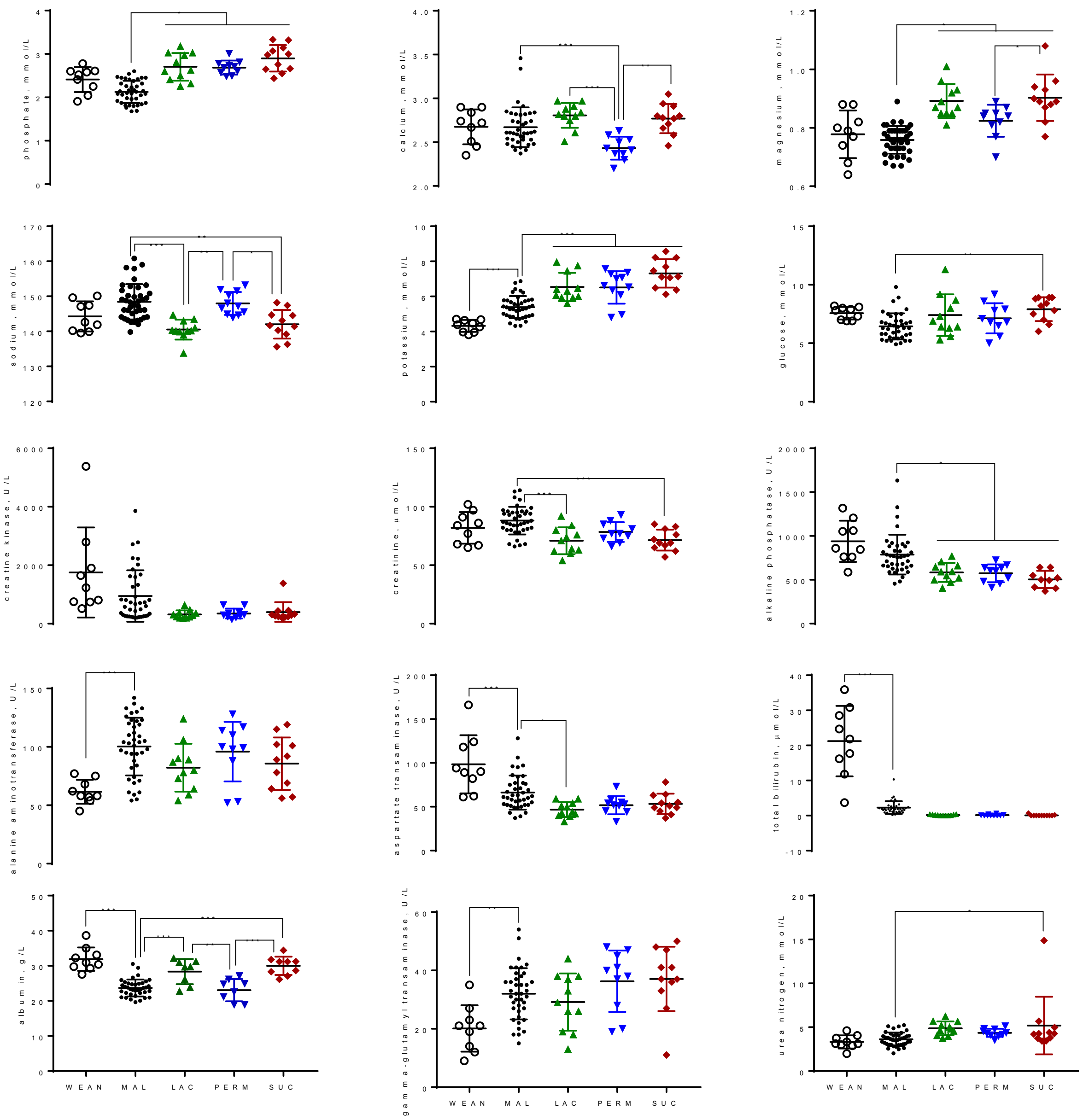

Figure 3. Plasma biochemical profile of newly weaned pigs (WEAN, n=10), and pigs fed a nutritionally suboptimal pure maize diet (MAL, n=40), or refed with corn-soy blends with added lactose (LAC, n=11), permeate (PERM, n=11) or sucrose (SUC, n=11). Panels indicate plasma concentrations of phosphate (A), calcium (B), magnesium (C), sodium (D), potassium (E), glucose (F), creatine kinase (G), creatinine (H), alkaline phosphatase (I), alanine aminotransferase (J), aspartate transaminase (K), total bilirubin (L), albumin (M), gamma-glutamyl transaminase (N), and urea nitrogen (O). All data are expressed as means ± std. err.

The notion that PERM may have increased plasma volume was also associated with an increased urine creatinine concentration in PERM versus LAC (tendency) and SUC, which is a surrogate marker of urine production. This observation suggests that urine production was lower in PERM relative to LAC and SUC. Likewise, the concentration of sodium, potassium, chloride and protein were elevated in PERM versus LAC an SUC, whereas urinary concentration of calcium, magnesium and urea was similar (Fig. 4).

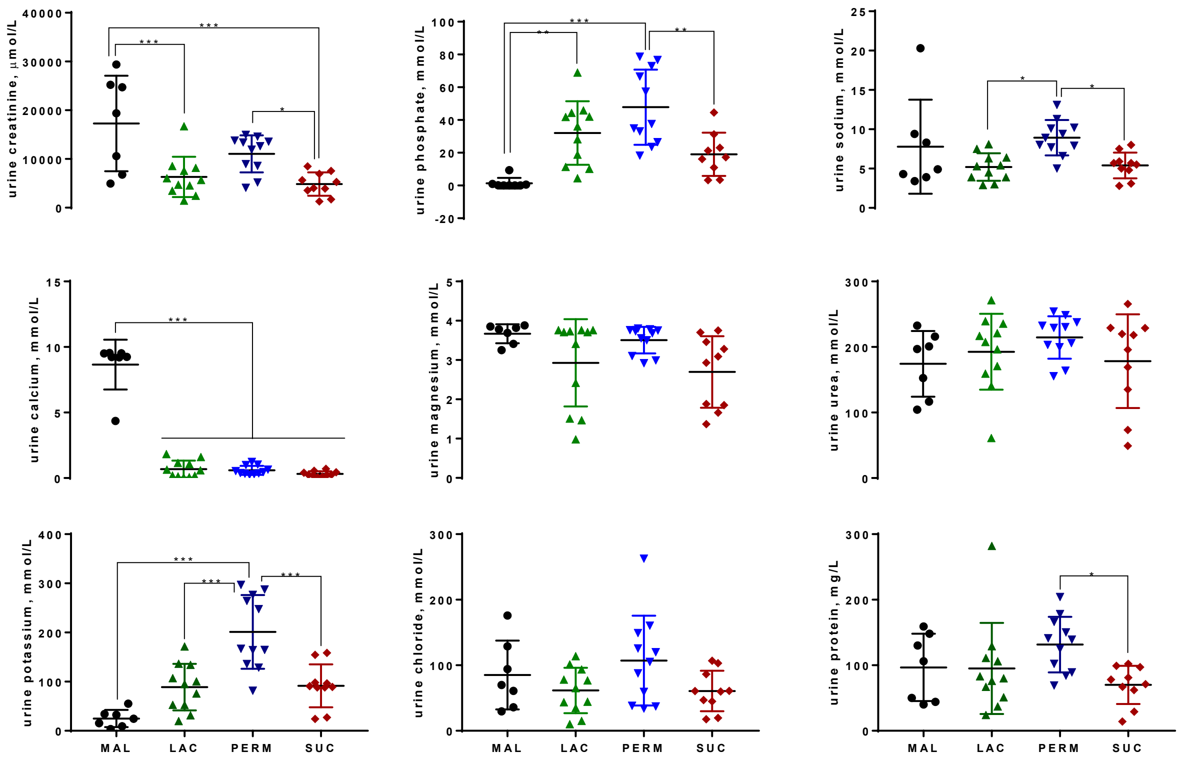

Figure 4. Urinary profile of pigs fed a nutritionally suboptimal pure maize diet (MAL, n=7), or refed with corn-soy blends with added lactose (LAC, n=11), permeate (PERM, n=11) or sucrose (SUC, n=11). Panels indicate urinary concentration of creatinine (A), phosphate (B), sodium (C), calcium (D), magnesium (E), urea (F), potassium (G), chloride (H) and protein (I). All data are expressed as means ± std. err.

Despite the marked effects of refeeding on growth and circulatory markers, this was not reflected in the activity of digestive enzymes in the gut mucosa. As expected, there were clear differences in activity between the small intestinal regions (for lactase, aminopeptidase N and A and dipeptidylpeptidase IV). But all enzymes were largely similar for MAL, LAC, PERM and SUC suggesting that the enzyme activity level at this stage of life and at this level of moderate malnutrition, was not susceptible to the dietary differences we formulated (Fig. 6).

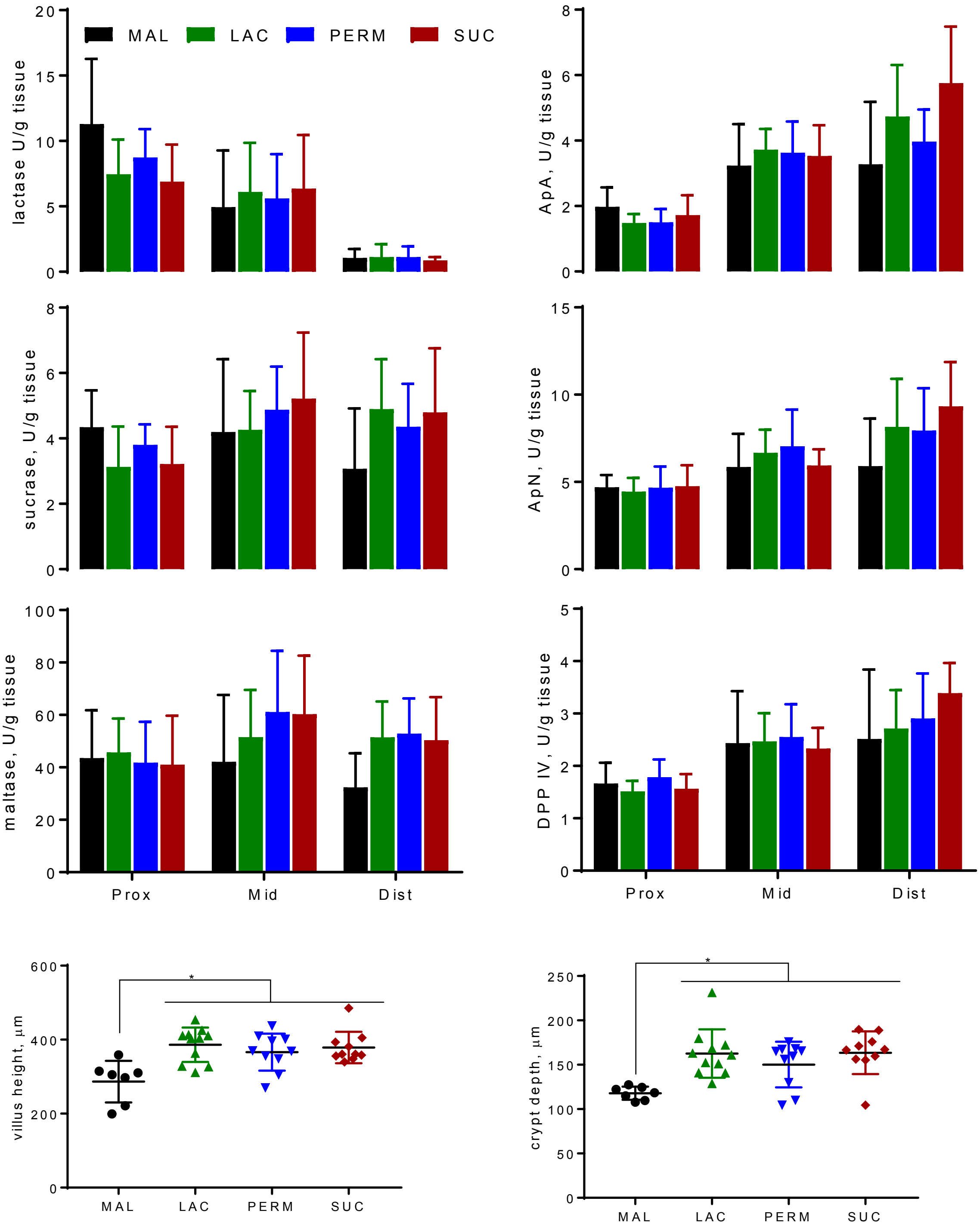

Figure 6. Mucosal digestive enzyme activity in pigs fed a nutritionally suboptimal pure maize diet (MAL, n=7), or refed with corn-soy blends with added lactose (LAC, n=11), permeate (PERM, n=11) or sucrose (SUC, n=11). Panels indicate regional (proximal, middle or distal) urinary concentration of creatinine (A), phosphate (B), sodium (C), calcium (D), magnesium (E), urea (F), potassium (G), chloride (H) and protein (I). All data are expressed as means ± std. err.

Likewise, we studied the microbiome in content from the proximal small intestine, and the rectum. In brief, we found that the microbial community, as indicated by the diversity, in the proximal small intestine and the rectum, were largely similar across all groups (Fig. 7). Even pigs in the MAL group showed a similar microbial profile relative to the three refeeding groups. Sub analysis of the diversity with the constraints of a multivariate redundancy analysis, did show a minor separation of MAL and SUC, whereas LAC and PERM grouped together (Table 3). However, relative to the total abundance of microbes, the observed discrepancy in low-abundant microbes is unlikely to play a key role.

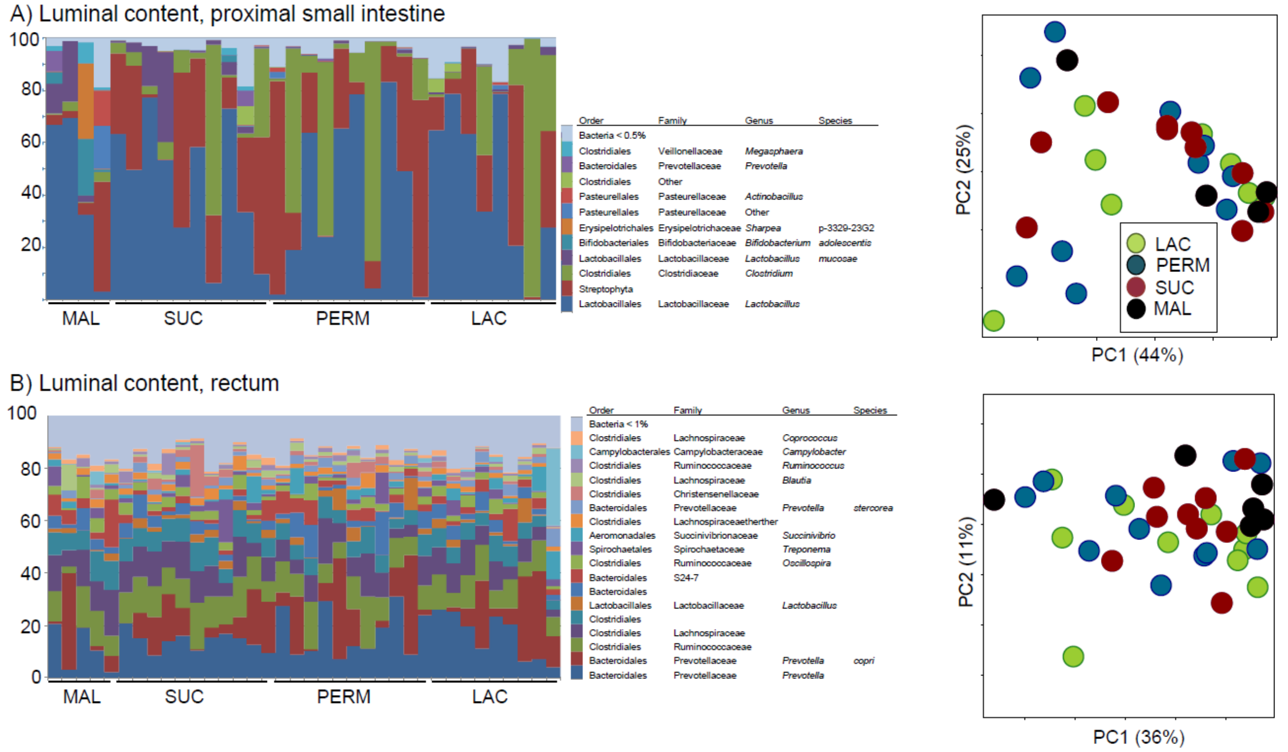

Figure 7. Gut microbiota (GM) composition. (A) Relative abundance of bacterial species as determined from 16S rRNA gene (V3-region) amplicons sequencing from stool samples. Right panel describes the lowest level of taxonomic annotation (B) Bray-Curtis distance based Principal Coordinate Analysis (PCoA) plot and (C) Redundancy Analysis (RDA) plot constrained by baseline and treatment groups (Lactose, Permeate and Sucrose). Analysis were based on 14,000 rarefied sequences per sample.

Table 3 Significant differences in mean distribution of bacterial species per treatment in pigs refed with corn-soy blends with either added sucrose (SUC), permeate (PERM) or lactose (LAC). Data represent probability values and different superscript letter within a row indicate significant difference.

				Mean distribution (%) per treatment*		
Order	Family	Genus	Species	SUC	PERM	LAC
Actinomycetales	Nocardiaceae	*Rhodococcus*	*fascians*	0.01[a]	<0.01[b]	<0.01[b]
Rickettsiales	Unclassified			0.02[a]	<0.01[b]	0.01[b]
Erysipelotrichales	Erysipelotrichaceae	Other		0.04[a]	0.01[b]	0.01[b]
Erysipelotrichales	Erysipelotrichaceae	Unclassified		0.06[a]	0.03[ab]	0.02[b]
Clostridiales	Christensenellaceae	Unclassified		4.25[a]	1.66[a]	1.05[a]

(*) Treatment associated bacteria (summarized to species level) were determined through sparse RDA, while significant differences ($p < 0.05$) were evaluated using one-way ANOVA and Tukey's HSD.

DISCUSSION

Permeate, lactose and sucrose in a corn-soy based diet for nutritional rehabilitation, support similar recovery in terms of voluntary feed consumption, growth velocity and body composition. While these clinical anthropometric measures were similar, there were clear discrepancies at the para-clinical level, particularly regarding plasma volume and urine production. As body weight and body composition were similar between PERM, LAC and SUC, the observed dilution of plasma-markers in PERM relative to LAC and SUC, collectively suggests that fluid has shifted from the extra-vascular to the intra-vascular space. Sodium concentration in plasma and urine was higher in PERM relative to SUC and LAC, reflecting the higher sodium intake from the permeate product. Frankowski et al. (2014) analyzed 18 different permeate products and found an average sodium content of 0.98% (range 0.7 – 1.33) in permeate dry matter. On average across the refeeding period this corresponds to an extra daily sodium intake of 0.25 kg feed x 100 g permeate/kg feed x 0.98% = 245 mg in PERM relative to LAC and SUC. The higher sodium intake presumably provided the expansion of plasma volume as indicated by lower erythrocytes and albumin in PERM relative to LAC and SUC. As the most dominant cation in the extracellular space, sodium is the most important determinant of fluid homeostasis. Plasma sodium levels are under strict control via the renin-angiotensin-aldosterone system and atrial natriuretic peptides, involving key organ systems (kidney, lungs, pituitary and heart). Provided that kidney-, lung-, pituitary- and heart function are normal, plasma sodium levels are controlled effectively to prevent both hypo- and hypertension. However, the current extra sodium intake in the PERM group did not associate with a higher urine excretion. On the contrary, urine creatinine levels, a surrogate marker of urine production, was higher in PERM relative to LAC and SUC, indicating lower urine production, confirming the higher concentration of electrolytes and protein. One interpretation of this finding is higher fluid retention in PERM, which also contributes to the observed plasma expansion. Although this is a plausible interpretation, it cannot be concluded with certainty as fluid intake was not measured and therefore may potentially have differed between the groups. Viart (1977a) studied malnourished infants at the time of hospital admission and found lower blood volume, blood pressure and cardiac output relative to a more normal reference group of infants. They found a general lowering of blood pressure with increasing degree of hypoalbuminaemia, and at albumin levels lower than approximately 15 g/L this led to cases of circulatory failure and death. In the current study, the pigs were only subjected to moderate malnutrition and we saw no indications of circulatory failure. Interestingly, in pigs of similar age, we have previously shown that progressive malnutrition was associated with low levels of fluid-regulating peptide hormones during the first three weeks of malnutrition, followed by a marked increase in the following weeks as their malnutrition progressed (Fabiansen et al., 2015). This increase may be viewed as an adaptive response, to increased sodium excretion in urine, thereby reducing blood pressure. Judging from the data of Viart (1977a, b) this may however lead to low blood pressure and circulatory failure if malnutrition progresses from moderate to severe. In another study Viart (1978) found that during realimentation of moderately malnourished infants, there was a lowering of hematocrit because the expansion of plasma was more rapid than the increase in erythrocyte volume. In contrast, we found that realimentation did in fact increase red blood cell number per litre of blood, but mostly so for LAC and SUC whereas PERM showed the before-mentioned sodium-driven dilution of the blood.

Collectively, realimentation with high-sodium products like permeate may be indicated during conditions of low blood volume and low levels of sodium. Caution must however be taken in severely malnourished patients that present with heart or kidney failure where control of sodium excretion and plasma volume has been deranged. The changes in concentration of circulatory markers, particularly electrolytes, appeared to be driven by the mineral fraction of the permeate product as this was not seen the group given lactose only. Moreover, for the majority of markers (circulatory and anthropometric), we saw no difference between LAC and SUC. Likewise, for endpoints related to the digestive system we saw very similar mucosal dimensions (villus height and crypt depth) and mucosal activity levels for sucrase, maltase, lactase and aminopeptidases between LAC, PERM and SUC. Similarly, the microbiome in the small intestine was similar across all groups, indicating that the influence of the base diet (i.e. corn-soy blend, accounting for 90% of the diet), was larger than the influence of the added sucrose, lactose and permeate accounting for 10% of the diets).

We conclude that refeeding after moderate malnutrition with a corn-soy based diet with added permeate, expands the plasma volume and reduces urine production relative to diets enriched with sucrose or lactose. For patients with low blood pressure, but normal heart and kidney function, expansion of plasma volume can be regarded as a benefit to support perfusion in peripheral tissues.

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11. Deviations

11.1 Scientific

For local logistic reasons, we had to omit one of the proposed refeeding groups (i.e. a group refed with corn-soy with added milk minerals). Given the number of animals we wanted in each group, it was not feasible to house more than three refeeding groups, due to the housing capacity of the experimental facility. Furthermore, we replaced some of the proposed endpoints (gut peristalsis, plasma citrulin and galactose, liver triglycedride, urinary mannitol and lactulose) with other endpoints which were judged to be of bigger value (ex vivo cytokine response to stimulation with TLR agnoists, gut microbial analysis).

11.2 Financial

No financial deviations.

11.3 Timetable

There was an initial delay from January 2015 to September 2015. A full-length draft manuscript has been produced, final publication is pending.

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

The most important effects of permeate during nutritional rehabilitation after moderate malnutrition appear to be driven by the mineral fraction rather than the lactose fraction. It is relevant to the dairy industry to demonstrate and document the effects of milk minerals during nutritional rehabilitation to create enough scientific base for inclusion of permeate as a supplement in rehabilitations diets. It is particularly important in future studies to assess the isolated effects of the mineral fraction of permeate, and how it compares with other commercially available mineral supplements. Also, it will be important to design studies where fluid balances in response to refeeding with permeate can be studied in detail (i.e. fluid, intake and output and fluid exchange between the intracellular, interstitial and plasma compartments). Clinical endpoints during refeeding, including blood pressure, tissue perfusion, cardiac and renal function will be important to assess.

13. Communication and knowledge sharing about the project

A draft full manuscript has been produced and an abstract has been published in the proceedings for ESPGHAN (European Society for Pediatrics, Gastroenterology, Hepatology and Nutrition), Geneva, May 2018. The abstract was selected for oral presentation at the conference.

14. Contribution to master and PhD education

Guest researcher Prof. Chris Van Ginneken, Antwerp, Belgium and post doc Frederik Hansen designed the ex vivo peristalsis assay.

PhD student Shuqiang Ren analyzed feed consumption data and evaluated mucosal dimensions histologically.

Master student Sinead O'Ferrall and Post doc Josue.L. Castro-Mejía analyzed the gut microbiome.

15. New contacts/projects

The project was carried out within the frame of already existing collaborations. For potential future studies of fluid transport, we will need to establish new contacts.

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: 30 October 2018 Signature, Project manager: Thomas Thymann

Date: 30 October 2018 Signature, DDRF-representative: Grith Mortensen

Appendix

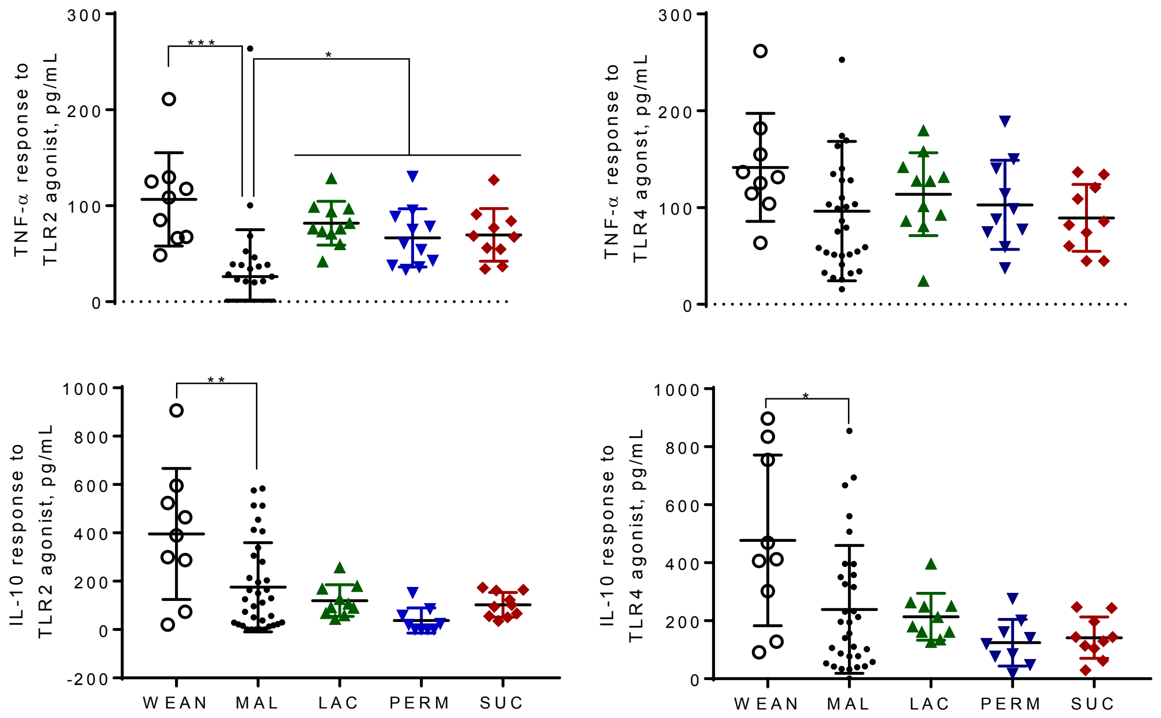

Figure 5. Cytokine response to ex vivo stimulation of leucocytes with TLR2 and TLR4 agonists. Blood samples were collected from newly weaned pigs (WEAN, n=10), and pigs fed a nutritionally suboptimal pure maize diet (MAL, n=40), or refed with corn-soy blends with added lactose (LAC, n=11), permeate (PERM, n=11) or sucrose (SUC, n=11). Panels indicate TNF-α response to TLR2 stimulation (A), TNF-α response to TLR4 (B), IL-10 response to TLR2 stimulation (C), and IL-10 response to TLR4 stimulation. All data are expressed as means ± std. err.