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Mark Manary:

Effekten af valleproteinkvalitet på behandling af moderat underernærede børn

The effect of dairy protein quality on treatment of moderately malnourished children

Mejeribrugets
ForskningsFond

September 2020

Final report

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

1. Title of the project

US: The effect of dairy protein quality on treatment of moderately malnourished children.

DK: Effekten af valleproteinkvalitet på behandling af moderat underernærede børn.

2. Project manager

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4. Sources of funding

The Danish Dairy Research Foundation (DDRF)
Arla Foods Ingredients p/s
Project Peanut Butter

5. Project period

Project period with DDRF funding: January 2017 – December 2019

6. Project summary

In Danish:
Målet var at sammenligne effektiviteten af en proteinkvalitetsoptimeret klar-til-brug supplerende diæt (RUSF) med en isonitrogen-kontrol RUSF i behandlingen af moderat, akut underernæring (MAM). For at nå dette mål gennemførte vi et randomiseret, kontrolleret, dobbeltblindet, klinisk effektivitetsundersøgelse. Proteinkvalitet henviser til en numerisk score, som tildeles en fødevare på basis af dens aminosyresammensætning. Fødevarer, hvor alle aminosyrerne forekommer i præcis de mængder, som kroppen har behov for, får den højeste score. Begge fødevarer indeholdt> 7% mejeriprotein, men det proteinoptimerede RUSF havde en beregnet fordøjelig uundværlig aminosyrescore (DIAAS) på 95%, mens kontrol-RUSF havde en beregnet DIAAS på 63%. 1737 børn i 6-59 måneders alderen fra Malawis landdistrikter blev behandlet med 75 kcal/kg/dag af enten kontrol- eller proteinkvalitet-optimeret RUSF i op til 12 uger. Der var ingen forskel i andelen af børn, der kom sig efter MAM mellem gruppen, der modtog proteinoptimeret RUSF (759/860, 88%) og gruppen, der modtog kontrol RUSF (766/877, 87%, forskel 1%, 95% CI -2,1 til 4,1, P = 0,61). Der var ingen forskelle i tid til bedring eller gennemsnitlig vægtøgning; der blev heller ikke rapporteret om bivirkninger. Begge RUSF'er gav meget ensvisende kliniske resultater, med restitutionshastigheder højere end normalt set i behandling for MAM. Efter forsøgets igangsætning opstod der en mulighed for at måle DIAAS for disse to RUSF'er. Måling af proteinkvalitet udføres ved hjælp af en svinemodel, hvor svinene fordres med testfødevarer, og hvor prøver fra svinetarmen udtages for at måle, hvor meget af hver aminosyre, der absorberes. Proteinkvaliteten af den optimerede RUSF var ganske uventet mindre end for kontrol-RUSF; DIAAS = 82% for den proteinkvalitets-optimerede RUSF og 96% for kontrol RUSF. Forskellen fra 82% til 96% gør, at man kunne forvente at se en klinisk forskel i vægtøgning eller helbredsmæssig bedring, hvis proteinkvaliteten var biologisk vigtig. De kontrollerede betingelser for dette forsøg antyder, at proteinkvalitet i supplerende fødevarer til MAM ikke kan benyttes til at få en uafhængig forudsigelse af klinisk effektivitet.

In English:

The aim was to compare the effectiveness of a protein quality optimized ready-to-use supplementary food (RUSF) to an isonitrogenous control RUSF in the treatment of moderate acute malnutrition (MAM). To achieve this aim we conducted a randomized, controlled, double-blinded, clinical effectiveness trial. Protein quality refers to a numerical score assigned to a food that is determined by its amino acid composition; foods which all of the amino acids in amounts that correspond to amounts used in the body have the highest score. Both foods contained > 7% dairy protein, but the protein optimized RUSF had a calculated digestible indispensable amino acid score (DIAAS) of 95% while the control RUSF had a calculated DIAAS of 63%. There were 1737 rural Malawian children 6-59 months of age treated with 75 kcal/kg/day of either control or protein quality optimized RUSF for up to 12 weeks. There was no difference in the proportion of children who recovered from MAM between the group that received protein optimized RUSF (759/860, 88%) and the group that received control RUSF (766/ 877, 87%, difference 1%, 95% CI -2.1 to 4.1, *P*=0.61). There were no differences in time to recovery or average weight gain; nor were adverse effects reported. Both RUSFs showed indistinguishable clinical outcomes, with recovery rates higher than typically seen in treatment for MAM. After the trial started, an opportunity arose to measure the DIAAS of these two RUSFs. Measurement of protein quality is done using a pig model, where pigs are fed the test food and samples from the

pig intestine are taken to measure how much of each amino acid is absorbed. Unexpectedly the protein quality of the optimized RUSF was less than the control RUSF; DIAAS = 82% for the protein quality optimized RUSF and 96% for control RUSF. The difference between 82% and 96% is such one would expect to see a clinical difference in weight gain or recovery if protein quality was biologically important. The controlled conditions of this trial suggest that in supplementary food products for MAM, protein quality is not an independent predictor of clinical effectiveness.

7. Project aim

DK: Undersøge effektiviteten af et mælkeprotein- kvalitetsoptimeret supplerende fødevareprodukt sammenlignet med et standard fødevareprodukt til behandling af moderat akut underernæring.

US: To test the effectiveness of a protein quality optimized dairy containing supplementary food compared to a standard food in the treatment of moderate acute malnutrition.

8. Background for the project

The annual incidence of moderate acute malnutrition (MAM) is 15% of all children in sub-Saharan Africa. Children are especially vulnerable in their first few years of life when their energy demands are highest. Impairments of their immunity from MAM make children susceptible to infectious insults, which further compromises their nutritional status. Reduced cognition from MAM may lead to chronic deficits, resulting in a lifetime of decreased productivity and economic earnings, which contribute to the cycle of poverty. Up to 10.2% of infant mortality can be attributed to MAM.

While there is no widely implemented management protocol for MAM, several supplementary food products, including lipid-based ready-to-use supplementary foods (RUSFs), have been formulated and successfully used. RUSF has been shown to increase recovery rates and decrease time to recovery when compared with corn soy blends.

The optimal protein composition of RUSF is uncertain. Quantifying only the total protein content in supplemental feeding can overestimate the amount of utilizable protein. Protein quality is a measurement that aims to quantify the capacity of a food to meet one's essential amino acid (AA) requirements based on physiologic status and body size. The quality of a protein is determined by assessing its essential AA composition as well as the digestibility of its AAs.

Protein and AA content in supplementary food products is vital, since the recipients receiving supplementary foods often have no or limited access to high quality protein and different physiologic needs than a healthy population. Studies have shown the addition of high-quality proteins, such as dairy proteins, to supplementary foods results in higher recovery rates and improved growth when compared to foods with only plant protein sources. One study found that a whey RUSF led to better rates of recovery than a soy protein RUSF despite the whey RUSF protein providing 33% less overall total protein content. It is not known what component of whey RUSF accounted for the better recovery rates, whether it was attributable to the higher protein quality, the presence of bioactive peptides, dairy's action as a prebiotic, or a combination of these. Regulating agencies are turning to protein quality metrics in their guidelines to standardize RUSF composition. However, gaps remain for the optimal quality, quantity, and source of protein to be included

in supplementary foods and to our knowledge, no prior clinical trial has compared two RUSFs with differing protein quality, but both with high amounts of dairy protein.

The aim of this clinical trial was to compare the effectiveness of a protein quality optimized RUSF (HiPro-RUSF) to an isonitrogenous control RUSF (C-RUSF) in the treatment of MAM. The hypothesis was that recovery from MAM of children receiving HiPro-RUSF would be superior to that for children receiving the C-RUSF.

9. Sub-activities in the entire project period

	Month																												
Activity	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Create & utilize a spreadsheet-based tool to calculate DIASS in different RUSF recipes that would include any available powdered dairy products containing substantial amounts of protein.																														
Use LP Tool for cost-minimize these recipes and maximize the DIASS.																														
Prepare these RUSFs in the food laboratory at WUSTL, and on the basis of organoleptic properties and physical properties, determine which are feasible																														
Conduct an acceptability trial of 2 of the most acceptable recipes. Choose the milk-RUSF.																														
Prepare to conduct the clinical trial, get approvals and develop protocols.																														
Conduct the clinical trial. Follow-up will continue until 3 months after the child has recovered.																														
Data analyses and report writing.																														
Dissemination																														

10. Deviations

Not an intentional deviation but after the commencement of the clinical trial, the opportunity arose to measure the DIAAS in a pig model. Eight pigs had a T-canula installed in the distal ileum to allow for sampling of ileal digesta. Pigs were then fed the C-RUSF, the HiPro-RUSF or a nitrogen-free diet for 7 days and the results were surprising. Calculation of DIAAS in the study foods and measurement of DIAAS in an intricate animal model varied considerably. For the calculated DIAAS, HiPro-RUSF was 92% and C-RUSF was 63%; for the measured DIAAS HiPro-RUSF was 82% and C-RUSF was 96%. If DIAAS in the supplementary food was a predictor of growth in recovery from MAM, we would not expect growth rates to be as similar as we observed. If the measured DIAAS in RUSF was a determinant of growth, the C-RUSF would have been superior. Thus, our hypothesis that protein quality is a characteristic of supplementary food that determines growth is not supported by these data.

11. Project results

The results have been published in Roediger R, Stein H-H, Callaghan-Gillespie M, et al. Protein quality in ready-to-use supplementary foods for moderate wasting. Matern Child Nutr.2020;16:e13019.https://doi.org/10.1111/mcn.13019 The figures and tables presented below are all results from that publication.

A total of 860 children were enrolled in the HiPro-RUSF group and 877 children in the C-RUSF group from June 2018 until March 2019(Table 5). Similar demographic, anthropometric and social characteristics were seen in the groups at enrolment, as one would expect with randomization (Table 5). Among children receiving HiPro-RUSF and C-RUSF, 759/860 (88%) and 766/877 (87%) recovered, respectively (difference 1%, 95% CI, −2.1 to 4.1, $p = 0.6$) (Table 6). No significant adverse events attributable to the RUSFs were found. The average time to recovery was 28.8 ± 19.4 days for the HiPro- RUSF and 28.6 ± 18.7 days for the C-RUSF ($p = 0.86$, Figure 1a). The rate of weight gain over the duration of feeding was 2.44 ± 2.31 g/kg/day for HiPro-RUSF and 2.41 ± 2.48 g/kg/day for C-RUSF ($p = 0.82$, Figure 1b). No variables apart from the enrolment MUAC and HAZ were found to be risk factors for failure to recover from MAM.

TABLE 5 Enrolment characteristics of children[a]

	Protein quality-optimized RUSF $n = 860$	Control RUSF $n = 877$
Age (months)	16.7 ± 10.77	16.1 ± 9.89
Under 2 years	698 (81%)	720 (82%)
Sex (F)	517 (60%)	533 (61%)
Enrolment MUAC (mm)	12.2 ± 0.42	12.2 ± 0.43
Enrolment weight (kg)	7.28 ± 1.5	7.22 ± 1.4
Enrolment height (cm)	70.9 ± 8.7	70.6 ± 8.2
Enrolment WHZ	−1.68 ± 0.8	−1.65 ± 0.78
Enrolment HAZ	−2.71 ± 1.3	−2.71 ± 1.37
Enrolment WAZ	−2.75 ± 0.77	−2.73 ± 0.8
Mother deceased	10 (1%)	18 (2%)
Father deceased	10 (1%)	22 (3%)
Father in home	677 (79%)	694 (79%)
Child breastfed	621 (72%)	650 (74%)
Number of siblings	2.09 ± 1.9	2.11 ± 1.89
Number of siblings deceased	0.30 ± 0.13	0.35 ± 0.13
HFIAS		
• Food secure	8 (1%)	5 (1%)
• Mildly food insecure	7 (1%)	9 (1%)
• Moderately food insecure	115 (14%)	114 (13%)
• Severely food insecure	717 (85%)	741 (85%)

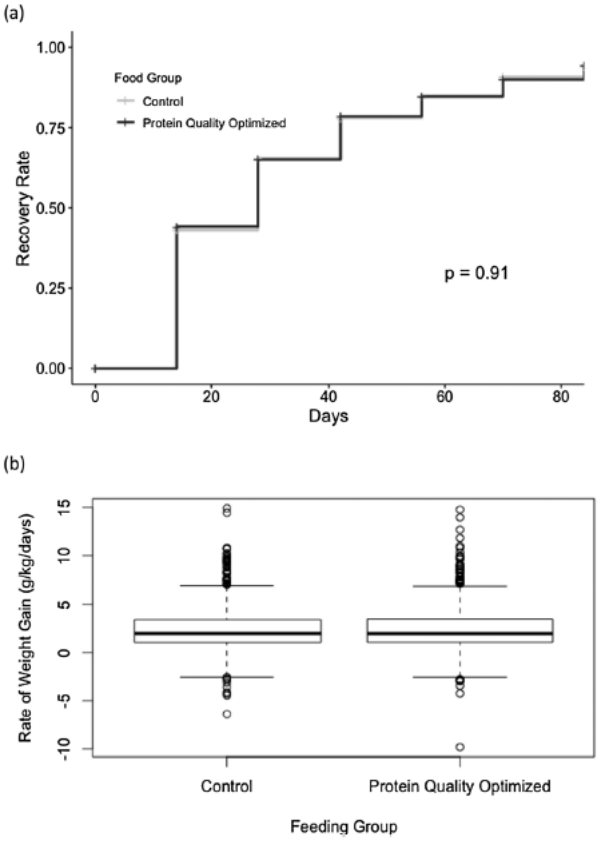

FIGURE 1 Recovery and weight gain in children with moderate acute malnutrition (MAM) receiving either protein quality-optimized or control ready-to-use supplementary food (RUSF). (a) Time-event plot of recovery from MAM by RUSF group and (b) rate of weight gain by food group. Boxplot presents median value as dark line, interquartile range as the box, minimum and maximum values as the whiskers and outliers are represented as dots ($p = 0.78$)

TABLE 6 Primary and secondary outcomes calculated using Fisher's exact test for categorical variables and Student's *t* test for continuous variables[a]

	Protein quality-optimized RUSF *n* = 860	Control RUSF *n* = 877	Difference (95% CI)	*p* value
Recovery from MAM	759 (88%)	766 (87%)	1% (2.1% to 4.1%)	0.61
Develop SAM	39 (4.5%)	45 (5.1%)	−0.6% (−2.6% to 1.4%)	0.58
Remain MAM at 12 weeks	38 (4.4%)	40 (4.6%)	−0.2% (−2.15% to 1.7%)	0.91
Lost to follow-up	24 (2.8%)	26 (3.0%)	−0.2% (−1.8% to 1.4%)	0.89
Rate of weight gain at 2 weeks (g/kg/day)	2.22 ± 2.8	2.26 ± 2.9	−0.04 (−0.23 to 0.31)	0.68
Rate of weight gain at outcome (g/kg/day)	2.44 ± 2.3	2.41 ± 2.5	−0.03 (−0.24 to 0.19)	0.82
Average time to recovery (days)	28.8 ± 19.4	28.6 ± 18.7	0.18 (−2.10 to 1.75)	0.86
Average weight gain (kg)	0.40 ± 0.3	0.40 ± 0.3	0.002 (−0.033 to 0.029)	0.90
Average MUAC gain (mm)	2.53 ± 3.0	2.47 ± 3.0	0.06 (−0.36 to 0.22)	0.64

Abbreviations: CI, confidence interval; MAM, moderate acute malnutrition; RUSF, ready-to-use supplementary food; SAM, severe acute malnutrition.
[a]Values are mean ± SD or number (percentage).

Analysis of the study foods with the porcine model revealed a measured DIAAS of 82% for the HiPro-RUSF with sulphur AAs being the limiting AA and 96% for C-RUSF with histidine being the limiting AA. The children in this study continued to eat their regular maize-based diet. Taking into account the AA contributions of maize allows evaluation of the limiting AA of the child's total intake, not just that of the supplementary foods. When AAs of 125 g of maize are included, the DIAAS for the HiPro-RUSF was 66% and for the C-RUSF was 73%; lysine was the limiting AA for both diets (Table 7).

TABLE 7 DIAAS for each amino acid (mg/g of each AA divided by reference pattern for catch-up growth in Table 1)

Essential amino acid	HiPro-RUSF calculated	C-RUSF calculated	HiPro-RUSF measured	C-RUSF Measured	HiPro-RUSF measured + maize	C-RUSF measured + maize
Histidine	1.02	0.63	1.02	0.96	0.95	0.91
Isoleucine	1.18	1.24	1.28	1.80	0.97	1.19
Leucine	1.09	0.99	1.10	1.38	1.08	1.22
Lysine	0.92[a]	0.96	0.98	1.15	0.66	0.73
Threonine	0.95	1.19	0.95	1.54	0.76	1.03
Valine	1.03	0.89	1.13	1.30	0.91	0.98
Sulphur AA	0.92[a]	1.17	0.82	1.25	0.90	1.12
Aromatic AA	1.22	1.12	1.33	1.30	1.08	1.05
Tryptophan	1.07	1.12	1.21	1.72	0.82	1.02

Abbreviations: AA, amino acid; C-RUSF, control RUSF; DIAAS, digestible indispensable amino acid score; HiPro-RUSF, protein quality-optimized RUSF; RUSF, ready-to-use supplementary food.
[a]Lysine was 0.921 and SAA was 0.923, so lysine was the limiting AA. However, in the pig data, when we use the new reference pattern for catch-up growth, the SAA is now the least abundant/digestible AA.

Protein quality scores of RUSF are ambiguous, contingent upon assumptions of ingredient AA content and not reflective of overall diet (Table 7). In this study, we found that protein quality score of the RUSF does not correlate with clinical recovery from MAM. Our RUSFs provided the same amount of total protein content, and both had high amounts of dairy protein but differed in protein quality score when measured. However, both delivered excellent clinical outcomes, as seen in the high rate of recovery from MAM. Protein quality scores are flawed because they do not differentiate between the relative importance of different AAs in growth or the contributions of the colonic microbiota to essential AA. The needed AA contribution from RUSF also depends on the AA contribution from the supplemented foods because it is the AA composition of the total daily intake that determines the adequacy of an individual's daily AA intake. It is, therefore, unlikely that a defined DIAAS or PDCAAS score will optimize the AA composition of all RUSFs.

Based on these findings, we would recommend against a specific protein quality being used to set guidelines to standardize RUSF.

The study population was rural African children aged 6-59 months who developed acute wasting in conjunction with household food insecurity. Extension of our findings to children from other demographics, or children with chronic illnesses is not warranted. This was not a study comparing dairy protein to vegetable protein. Our data do not inform the nutrition community about the suitability of substitutions of dietary plant protein for dairy in supplementary food products. Looking ahead, we recommend further research into the effect of different protein sources on clinical outcomes when using supplementary food products. We encourage caution when building recommendations for supplementary food products around protein quality scores, given the lack of high-quality clinical trial evidence to suggest their validity.

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

Our RUSFs provided the same amount of total protein and both had high amounts of dairy protein but differed in protein quality score when measured. Both delivered similar, excellent clinical outcomes, as seen in the high rate of recovery from MAM. Previously DDRF funded a comparison of dairy protein RUSF and vegetable protein RUSF, and dairy was superior. This current project, with some of the best outcomes seen worldwide for treatment of MAM, prove that dairy protein is superior, and the protein quality score **does not** affect outcome. This evidence solidifies the position of dairy protein as a preferred ingredient in food aid products. It is about the dairy origin of the protein that lends superiority, not the protein quality score.

13. Communication and knowledge sharing about the project

Papers in international journals:
Rebecca Roediger, Hans-Henrik Stein, Meghan Callaghan-Gillespie, Jeffrey Kahn Blackman, Kristin Kohlmann, Kenneth Maleta, Mark Manary (2020). Protein quality in ready-to-use supplementary foods for moderate wasting. Matern Child Nutr. 2020;16 :e13019. https://doi.org/10.1111/mcn.13019

Oral presentations at scientific conferences, symposiums etc.:
Rebecca Roediger MD, 'Dairy to vulnerable populations', Dairy Matters, Aarhus, November 11, 2019.

Oral presentations at meetings:
Rebecca Roediger, MD, GI 3rd Year Fellow, "Comparison of two Ready-To-Use Supplementary Foods (RUSF) in the treatment of moderate acute malnutrition (MAM)". Gastroenterology Division, Washington University, June 12, 2020.

14. Contribution to master and PhD education

N/A

15. New contacts/projects

Previous studies have found a beneficial effect of dairy protein in the treatment of MAM but there are conflicting reports regarding the importance of lactose. Further dairy-related projects should look at the comments of milk and allow for the separation of dairy protein from lactose to elucidate the beneficial effects of dairy protein and lactose alone and in combination on recovery from MAM and gut health. Through generous funding from DDRF we will study this relationship in our study "Milk Matters in Malnutrition." We have also discovered the ambiguousness of protein quality scores as they relate to RUSF and this can be used to inform future applications.

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: July 30, 2020 Signature, Project manager: _____

Date: 28 September 2020 Signature, DDRF-representative: _____