

Human milk analyser underestimated protein content of unfortified and fortified samples compared to elemental analysis

When premature infants are given human milk it needs to be fortified with bovine protein to provide all the essential nutrients they need, including protein.¹ The MIRIS human milk analyser (MIRIS AB, Uppsala, Sweden) analyses human milk at the bedside, but concerns have been raised about its accuracy.²

This prospective study was conducted from January 2012 to August 2012 at the Medical University of Vienna, Austria, and McMaster University, Hamilton, Canada. It showed how accurately MIRIS analysed fortified human milk by comparing it with elemental analysis, a new validated micro-method that precisely measures the protein content.³ The effect that pasteurisation had on the protein content was also determined. We used two frozen 24-hour pooled samples from each of five mothers. After thawing, one sample underwent pasteurisation and the other did not. All 10 samples were measured with MIRIS and elemental analysis. The study was approved by the local ethics committee in Vienna (EC Nr: 1121/2012), but informed consent was not required as the samples were surplus to requirements.

The samples were frozen at -21 to -27°C until needed, then thawed at $+4$ to $+6^{\circ}\text{C}$ in the refrigerator over 12 hours. One sample from each mother was pasteurised at 63°C for 30 minutes with the Barkey clinitherm pasteur XPT (Barkey GmbH, Leopoldshoehe, Germany). We measured the unfortified human milk, and then, we added 4.3 g/100 mL of Milupa Aptamil FMS (Danone GmbH, Friedrichsdorf, Germany) followed by Milupa Aptamil Protein+ (Danone GmbH, Friedrichsdorf, Germany) in eight 0.5 g steps, to a maximum of 4.0 g/100 mL. Both products are based on casein and whey protein hydrolysates. All the samples were divided into two aliquots and frozen again at -80°C . The first set was thawed and measured using the MIRIS human milk analyser in Austria. The second set was sent to Canada on dry ice and measured with elemental analysis.

MIRIS uses mid-infrared transmission spectroscopy.² All human milk samples were heated to 40°C and vortexed to homogenise all the components properly before each measurement. The elemental analyser was the Vario PYRO cube (Elementar Americas Inc, New Jersey, USA).³ It is based on the Dumas combustion principle, and its precision is comparable to the Kjeldahl method. Both devices were calibrated, and quality control checks were carried out before measurements.

The outcomes were expressed as medians and ranges. We compared the protein contents of pasteurised and unpasteurised samples using Kruskal-Wallis analysis of variance then the Mann-Whitney *U* Test. Analysis of variance with repeated measurements was used to identify differences in protein between the two methods. The analysis used SPSS version 20 (IBM Corporation, New York, USA).

Figure 1 shows that the protein content of the unfortified milk was different when measured by MIRIS (median 1.05 g/100 mL, range 0.90-1.40 g) and elemental analysis (median 1.22 g/100 mL, range 0.92-1.40 g). There was an impressive deviation of 19% between MIRIS and elemental analysis at the maximum fortification level. Repeated measurement analysis of variance provided significant results for the increase in protein concentration ($P < .01$) and elemental analysis ($P < .01$). Fortifying the human milk had a significant impact on group comparisons. Standard fortified human milk showed significantly lower protein values (median 1.85 g/100 mL, range 1.20-2.10 g/100 mL) with MIRIS than elemental analysis (median 1.98 g/100 mL, range 1.80-2.28 g/100 mL). As increasing protein was added, the difference between the two methods increased. For example, the protein difference was 0.98 g/100 mL when the maximum of 4.0 g protein per 100 mL was reached ($P < .01$). There were no significant differences between the pasteurised and unpasteurised samples in both groups ($P > .05$).

Fusch et al indicated that MIRIS underestimated the protein content in human milk by -0.2 g/dL and showed that device-specific correction factors and reconfiguration would provide reliable results.² One reason for the MIRIS gap could be the protein powder, which affects the human milk matrix. As a result, the fortified human milk was outside the confidence limits of the MIRIS calibration and could not be adequately measured. Another reason might be that the human milk became too viscous after fortification and, therefore, could not be measured correctly.

Manufacturers have launched human milk fortifier and protein powders that can be mixed with human milk without any data on compatibility checks or the bioavailability of single components.

We showed that pasteurisation had no impact on the protein content of human milk, in line with previously published data.^{4,5} It appears to preserve the biological activity of proteins and the amino acids remain stable. Nevertheless, a number of factors

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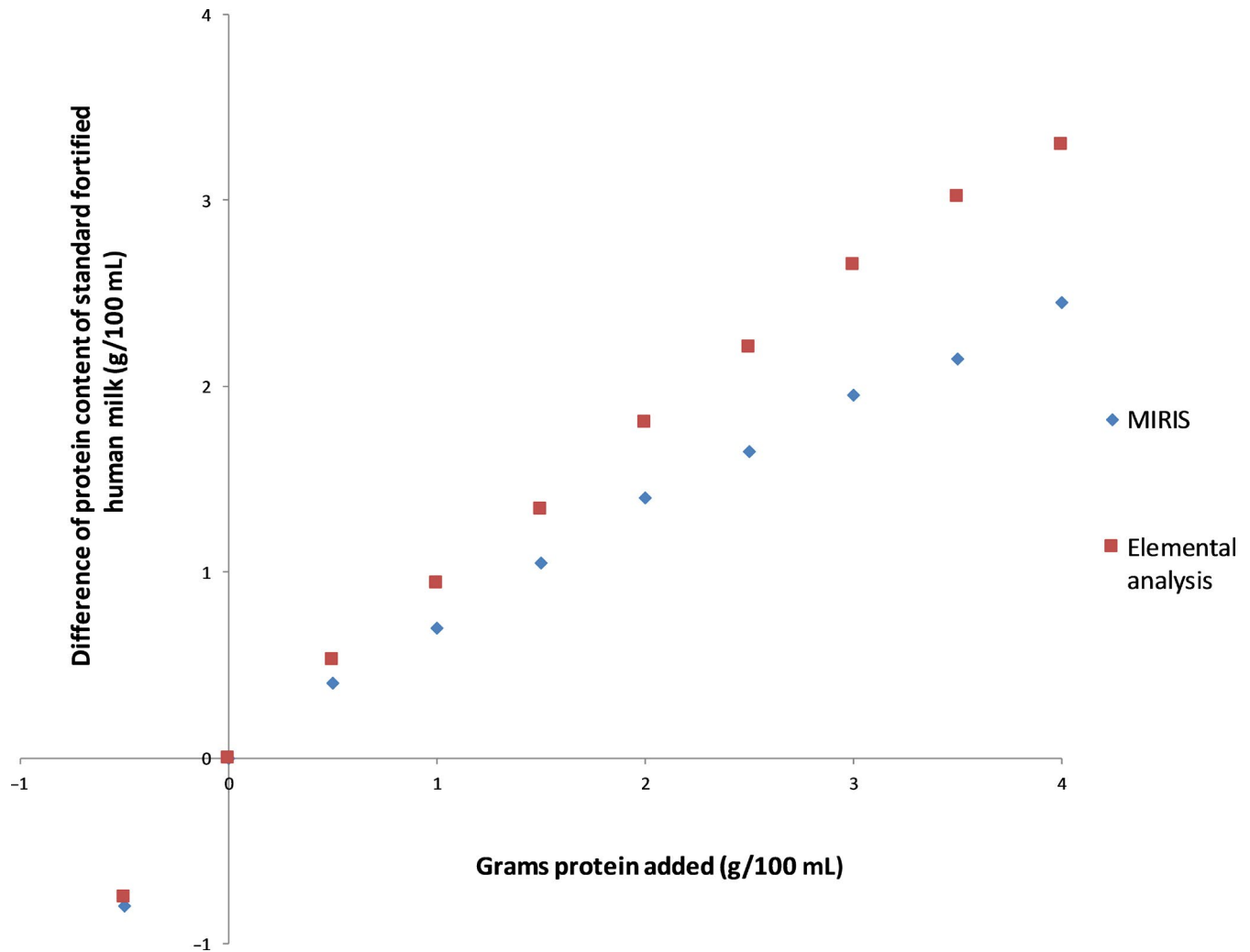


FIGURE 1 Protein levels of 10 samples, measured by MIRIS and elemental analysis for the different fortification steps. The first fortification step was reset to zero, and the fortification steps can be interpreted independently from the basic value. All human milk samples were measured in duplicate, and the values were averaged

might affect the outcome, like heating time, temperature, heating method and milk volume, as well as the type of human milk analyser. Our study was limited by the small sample size and further studies are needed to confirm our findings. The strength of the study was that we compared MIRIS to elemental analysis. We showed that MIRIS significantly underestimated the protein content of human milk compared with elemental analysis and was more inaccurate at higher protein fortification levels. Both methods revealed that pasteurisation had no effect on protein content.

CONFLICTS OF INTEREST

None.

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