Assessment of protein quality of infant milk food formula supplemented with lactulose

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To examine the protein quality of infant food formula supplemented with lactulose (bifidogenic factor), groups of 21-day male weanling rats were fed diet containing lactulose at 0.5% for 4 weeks. The gain in body weight of rats fed lactulose supplemented diet were similar to that of rats fed lactulose free and skim milk powder fed diet as controls. No difference in overall acceptability pattern was also observed in studies conducted with human subjects for organoleptic evaluation. The results suggest that supplementation of lactulose in infant food formula does not affect either the protein quality as indicated by protein efficiency ratio in rats as compared to control formulae or overall acceptability by human subjects.

Of late increasing attention is being paid on the use of non-digestible oligosaccharides as special nutrients in food formulations. Although lactulose (4-O-β-D-galactopyranosyl-D-fructose), an isomer of lactose (4-O-β-D-galactopyranosyl-D-glucose) and a non-digestible disaccharide of glucose and fructose, was synthesised by Montgomery and Hudson, its nutritional significance as a bifidogenic factor was not known until late fifties. Since then, lactulose has been successfully being used as a bifidogenic factor in infant food formulae.

At present, the amount of lactulose that is being used for the preparation of nutritional and pharmaceutical products is estimated at 6000 tonnes per year in the world. Lactulose has been approved as a special food material in Japan for health maintenance and protection against enteric diseases. Suzuki et al. and Nagendra et al. have reported higher retention of micronutrients, lower caecal pH and growth of bifidobacterial flora in the caecum of lactulose fed rats. Supplementation of lactulose (0.5%) in infant food formula resulted in increased growth of bifidobacterial flora in human subjects. Further lactulose administration has not been reported to cause any adverse effects and its safety is similar to that of sucrose. Adachi and Patton have reviewed the significance of lactulose in milk products and have reported that some milk mucoproteins may be physiologically important. Studies on the shelf-life of lactulose supplemented infant milk food formula have not shown any physicochemical changes. Thus, lactulose finds an important place as a food additive in the preparation of infant milk food formulations. Although bifidogenic nature of lactulose is known, limited data exist on the protein quality of infant milk food formulations supplemented with lactulose.

Therefore the present study focuses on the effect of supplementation of lactulose in infant milk food formulation on the growth rate as evidenced by food efficiency ratio (FER), protein efficiency ratio (PER) in animal studies and acceptability of the product by human subjects.

Lactulose preparation—Lactulose was prepared by the alkaline isomerisation method involving treatment of lactose solution containing calcium hydroxide. This resulted in 30% isomerisation of lactose to lactulose. The lactose-lactulose mixture was clarified with anionic (Indiana-80, BDH) and cationic (Amberlite-IR C50, BDH) resins and concentrated using rotovaporator. After removal of unreacted lactose by crystallisation at 4°C for 4 to 5 hr, a 50% syrup was obtained, wherein lactulose constituted one half of the sugar mixture. Lactulose content in the syrup was estimated according to the method of Nagendra and Venkat Rao. The syrup was diluted to desired concentrations prior to use.

Preparation of infant food formulae—Two batches of spray dried infant milk food formulae were prepared at Institute’s pilot plant either with (Formula II) or without (Formula I) lactulose supplementation as described earlier. The dried product was sealed under nitrogen and stored in containers at 4°C until
The proximate composition of the infant formula was determined as per AOAC procedures.  

Animal experiments—Groups of male weanling rats (Wistar-CFt strain, 35±2g) obtained from the animal house facility of the Institute were used. Animals statistically grouped by randomized design were assigned to 3 groups of 10 animals each. They were housed individually in stainless steel cages with screen bottom and maintained at 22±2°C, 60% RH and exposed to 12:12 hr L:D cycle. Group I and II were fed lactulose free and lactulose supplemented diets while group III was fed skim milk powder diet. Group I and III were treated as controls. The diets were prepared at 10% protein level (N X 6.25) according to Chapman et al. The animals had access to food and water ad libitum. Daily food intake and weekly body weights were recorded.

Organoleptic evaluation—The infant formulas were evaluated by a panel of eight judges for quality characteristics such as flavour, mouthfeel and overall acceptability on an 8 point Hedonic Scale like, 8-like extremely, 7-like very much, 6-like moderately, 5-like slightly, 4-dislike slightly, 3-dislike moderately, 2-dislike very much, and 1-dislike extremely.

- The proximate composition (%) of the infant formula I and II indicated levels of moisture 2.0 and 1.9; crude protein 12.2 and 12.1; total fat 20.0 and 20.0; ash 5.8 and 5.7; carbohydrates 60.0 and 60.1; lactulose, traces and 0.8 respectively. An additional amount of 0.17% lactulose was measured in formula II which was due to lactulose formation which occurs via alkali transformation of lactose by the Loblry-de-Bruyn and Alberda-van-Ekenstein mechanism. Traces (0.07-0.1%) of lactulose detected in the formula I may be due to the cumulative effects of heat treatment during various stages of processing. Andrews showed that lactulose formation occurred during UHT treatment, pasteurisation and storage of milk.

No significant difference either in food intake or gain in body weight were observed among different group of rats (Table I). Although food intake in the lactulose supplemented group (Gr II) was marginally low compared to group I and III, PER was, however, slightly higher indicating better palatability and food conversion. PER (Gr II) was also high and was comparable to that of group I and III. Marginally low gain in body weight in lactulose free diet (Gr I) may be attributed to low FER.

<table>
<thead>
<tr>
<th>Dietary groups</th>
<th>Food intake</th>
<th>Protein intake</th>
<th>Body wt gain</th>
<th>FER</th>
<th>PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr I</td>
<td>365±4.3</td>
<td>36.5±0.4</td>
<td>112±4.5</td>
<td>0.29</td>
<td>2.88</td>
</tr>
<tr>
<td>Gr II</td>
<td>340±4.1</td>
<td>34.8±0.4</td>
<td>112±4.5</td>
<td>0.37</td>
<td>3.27</td>
</tr>
<tr>
<td>Gr III</td>
<td>355±9.0</td>
<td>35.5±0.9</td>
<td>116±8.4</td>
<td>0.32</td>
<td>3.26</td>
</tr>
</tbody>
</table>

I—lactulose free infant formula; II—lactulose supplemented infant formula; III—skim milk powder diet (control); FER—Food efficiency ratio; PER—Protein efficiency ratio

Values are not significant between control and experimental groups (Student’s t test)

### Table 2—Organoleptic evaluation of infant food formulas with or without lactulose.

<table>
<thead>
<tr>
<th>Products</th>
<th>Flavour</th>
<th>Mouthfeel</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula I</td>
<td>6.63±0.2</td>
<td>6.63±0.2</td>
<td>6.63±0.2</td>
</tr>
<tr>
<td></td>
<td>(6-7)</td>
<td>(6-7)</td>
<td>(6-7)</td>
</tr>
<tr>
<td>Formula II</td>
<td>6.13±0.3</td>
<td>6.38±0.2</td>
<td>6.50±0.2</td>
</tr>
<tr>
<td></td>
<td>(5-7)</td>
<td>(6-7)</td>
<td>(6-7)</td>
</tr>
</tbody>
</table>

I—lactulose free infant formula; II—lactulose supplemented infant food formula

The higher PER (3.27) in Gr II was on par with the control SMP group (3.26), and is in conformity with the standards (>2.5) laid down by the Indian Standard's Institute for infant food formulas and also conforms with the specification of the Protein Advisory Group of the United Nations for infant foods. It is also reported that supplementation of lactulose in infant food formula does not affect the shelf life of the product and also increased the growth of bifidobacterial flora and reduced the colon pH in rats and human subjects. Further, feeding lactulose at 0.5% in infant formula to growing rats and human infants do not cause any side effects. Lactulose at higher concentrations (>1%) is reported to cause diarrhoea and weight loss in rats. Further, it is reported that 0.5-1% of lactulose does not affect absorption and retention of nitrogen, calcium, phosphorus and iron and also does not affect haematology, histology, serum and tissue chemical composition on continuous consumption during the growing period.
No differences were observed in the flavour, mouthfeel and overall acceptability between control and lactulose supplemented formula (Table 2). The rating of the product were almost similar. The acceptability pattern of the products was good with average scores for flavour, and mouthfeel being more than 5. The results show that addition of lactulose in infant milk food formula does not affect overall acceptability of the product.

Therefore, it can be concluded that supplementation of lactulose in infant food formulas does not affect protein quality as evidenced by gain in body weight, FER, PER and acceptability pattern. Further, lactulose as a food additive has high potential in many of the food products. Use of lactulose in food and pharmaceutical products in India and other developing countries is still in infancy. Hence, efforts have to be made for the use of lactulose as a food additive in infant food formulas and pharmaceutical products.

References
20 ISI, Indian Standards Institution, No 1547, New Delhi, India (1968).