Growth of infants born to HIV-positive mothers fed a whey-adapted acidified starter formula with prebiotics and nucleotides

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Keywords: milk formula; acidification; probiotics; nucleotides; infant growth

Abstract

Objectives: The objectives of this study were to evaluate whether infants born to known HIV-positive mothers, but who were not themselves infected with HIV and who were fed a chemically acidified starter formula with prebiotics with or without nucleotides during their first six months, displayed growth rates equal to uninfected infants fed a chemically acidified starter formula without prebiotics or nucleotides.

Design: The design was a multi-centre, double-blinded randomised controlled trial.

Setting: The study was carried out in four academic hospitals, three in Johannesburg and one in Cape Town, South Africa.

Subjects and intervention: The subjects were newborn infants born to consenting HIV-positive women who had previously decided not to breast feed. The infants were randomised to receive one of three milk formulas. The intervention comprised chemically acidified formula without prebiotics or nucleotides, with prebiotics only, or with prebiotics and nucleotides.

Outcome measures: The outcome measures were the growth parameters through the first six months of life.

Results: Of the 150 randomised infants, 50 did not complete the study and 16 (12.8% of those tested) were infected with HIV, leaving 84 infants available for analysis. All three formulas were tolerated well, with no differences in growth parameters seen with the addition of prebiotics and nucleotides. The growth rates of the study infants up to the age of six months were very good, showing an increase in Z-scores from negative values at the time of enrolment in the first week after birth to around zero for length and > 0.5 for weight.

Conclusions: The three chemically acidified formulas were tolerated well and resulted in good growth over the first six months of life. No benefits were seen with the addition of prebiotics or nucleotides. The growth rates were similar to those found in previous studies of ours on biologically acidified formulas. The chemical acidification of infant formulas appears to be a realistic alternative to biological acidification should an acidified formula be required.

Introduction

The optimal choice of feeding for an HIV-exposed infant is complex in developing countries. The risks of transmission of HIV through breastfeeding must be balanced against the many known benefits of breastfeeding in reducing infant mortality and morbidity. The cost of replacement feeding is substantial and also needs to be considered. Regardless of feeding choices, the growth of infants born to HIV-infected mothers is determined largely by whether the infant is infected with HIV. An overview by Isanaka et al[1] of patterns of postnatal growth in HIV-infected and HIV-exposed infants reviewed all papers published between 1985 and the end of 2008. While infants who were shown to be infected with HIV had poorer growth than those who were uninfected, those exposed to but uninfected with HIV had similar growth patterns to the reference population.[1]

For infants who are not breast fed from birth, a standard whey-adapted starter formula that is close in many respects to human milk is usually recommended. For a number of years a biologically acidified starter formula has been commercially available in South Africa and has been used extensively. There is some evidence that intestinal infections are reduced in infants fed a biologically acidified formula.[2] In a recent study, infants aged four to six months receiving the biologically acidified formula had the same number of diarrhoeal episodes, but they were less severe.[3] A recent systematic review on biologically acidified formulas concluded that the existing data were too limited to allow firm conclusions regarding their potential benefits and safety, and recommended that further clinical trials should be performed.[4]

Biological acidification is usually achieved by introducing lactic acid-producing bacteria during the production process. Acidification

can also be done chemically by adding L(+) lactic acid. This may have advantages in that production is simpler and cheaper and the precise amount of acidification can be controlled, leading to less variation from batch to batch. However, data on chemically acidified formulas in full-term infants have not been published.

Attempts to modulate the microbiota in formula-fed infants towards increased bifidobacteria counts have been achieved by adding either living bifidobacteria (probiotics) or substances enhancing the growth of lactic acid bacteria (prebiotics) to infant feeds. There is evidence that prebiotics may modulate the intestinal microbiota and the immune system in infants in the same way that human milk does.[5] Compared to control formulas, infants fed nucleotide-supplemented formula had an enhanced immune response to influenza and diphtheria, but not to tetanus or polio vaccinations,[6] and also had fewer diarrhoeal episodes than those fed a non-supplemented formula.[7,8]

The main objective of this study was to evaluate the growth of infants, born to known HIV-positive mothers but who were not themselves infected with HIV, fed a chemically acidified starter formula with prebiotics with or without nucleotides during their first six months. If their growth was satisfactory, this would then allow the evaluation of the safety of the formulas. At the time this study commenced, we could find no published data on the use of chemically acidified milks for feeding term infants who were well. Since we also were doing studies on infants fed standard and biologically acidified formulas, comparison of growth rates of infants fed chemically acidified formulas with infants from these studies could be made. The secondary objectives were to assess the infants' tolerance to chemically acidified formulas with and without prebiotics and nucleotides, to evaluate the frequency of episodes of morbidity (especially those related to infectious diseases), and to assess protein, biochemical and immunological status.

Methods

This study was a multi-centre, prospective, randomised, double-blind controlled trial. It was carried out at the Johannesburg, Chris Hani Baragwanath, and Coronation Hospitals in Johannesburg, and at Groote Schuur Hospital in Cape Town, South Africa.

All pregnant women attending the antenatal clinics of the hospitals are routinely offered testing for HIV. Those who test positive receive full post-test counselling, which includes discussion of the potential risks of breast- and formula feeding. During the study period, those who indicated that they did not plan to breast feed were then informed about the study. However, formal enrolment into the study only took place after delivery in order to ensure that all criteria for enrolment were met and that the mother still planned not to breast feed.

Inclusion criteria were healthy, normal, full-term infants (gestation 37 to 42 weeks and birth weight 2 500 to 4 200 g) born to HIV-positive women who had elected not to breast feed. Infants with major congenital abnormalities and those with major illnesses requiring

admission to an intensive care unit or hospital for more than three days after birth were excluded from the study. Subjects were also excluded if the mother planned to introduce feeds other than milk in the first four months of life, or if the mother planned to move out of the study area during the six-month study period. Recruitment into the study had to take place within one week of birth.

After enrolment, the infants were randomised into one of three feeding groups:

1. A chemically acidified whey-adapted starter formula (CAF)
2. A chemically acidified whey-adapted starter formula with prebiotics (CAFP)
3. A chemically acidified whey-adapted starter formula with prebiotics and nucleotides (CAFPN)

Acidification was achieved by the addition of L(+) lactic acid. The L(+) lactic acid constituted 2% of the powder formula and resulted in a pH of 4.7 when the product was reconstituted with tap water. The prebiotic was a blend of short-chain and long-chain fructo-oligosaccharides (70:30) with a total of 2 g per litre when reconstituted to liquid form. The nucleotides were a blend of cytidine monophosphate, uridine monophosphate, adenosine monophosphate and guanosine monophosphate (approximately 55:35:6:4 respectively). The formulas were similar to starter formulas in other respects, containing 67 kcal/100 ml, 1.5 g/100 ml of whey-adapted protein and standard concentrations of electrolytes, trace elements and vitamins. All formulas contained polyunsaturated long-chain fatty acids.

Assignment to the formula groups was performed by a computer-generated randomisation table using Excel. Block randomisation was performed for each centre separately by the sponsor. Each centre received a set of sealed envelopes containing the subject number and treatment code. Once an infant was enrolled, the next envelope in the sequence was opened by the study personnel, and this indicated which of three colour-coded tins of milk the subject should receive. The investigators and the participants were blinded to the formula assignments, and the colour-coded tins ensured blinding as well as continuity of supply of the same formula to each infant. Details of the formula composition did not appear on the tins, in keeping with the double-blind design of the study.

At the time of enrolment, basic information regarding the pregnancy and delivery were recorded and the study personnel measured the infant's weight, length and head circumference. Weight was measured to the nearest 10 g on an electronic scale, supine length was measured to the nearest 1 mm on a standard measuring board, and head circumference was measured to the nearest 1 mm using a standard non-elastic plastic-coated measuring tape. The electronic scales were calibrated by the supply company and the same equipment was used at each hospital for all subsequent anthropometric measurements. The study personnel at each hospital were trained in measurement techniques and remained constant during the course of the study. Follow-up visits were scheduled for 14, 28, 42, 56, 91, 119 and 182 days after enrolment.

After enrolment and randomisation to the study formula, the mothers received careful and detailed instructions regarding the safe preparation of formula and were advised according to standard guidelines regarding the usual amounts of milk required by their infants. However, the mothers were informed that their infants should be fed on demand, without restrictions regarding the frequency and volumes of feeds, and that additional water should not be given. The information on the safe preparation of formula was re-enforced at each visit. Formal follow-up visits were as listed above, but, for practical reasons (size and weight of tins of formula that could be taken on public transport), mothers generally returned more frequently to collect tins of the powdered formula, typically every two weeks. The supply of the study formula was continued until the end of the study period, when the infants were at the age of six months. Infants were considered not to have complied with the study protocol if any of the following occurred:

1. The mother gave more than one bottle of another formula per week
2. The infant did not receive the study formula (due to the mother not being able to get supplies or illness in the infant) for more than seven consecutive days
3. Significant amounts (> 20 g) of foods other than formula were introduced before four months of age
4. There was failure to complete one of the visits at 42, 119 or 182 days, since these visits were regarded as essential

Although these subjects were withdrawn from the study for future analysis, they were still offered the study formula for the six-month period of the study. This also applied to infants who tested HIV positive.

In addition to anthropometric measurements at each visit, the infants were examined by the study personnel and a record was made of any illness and the concomitant treatments. The caregivers were interviewed at each visit regarding interim health status and the number of times the infant had been seen at any health-care facility since the last visit. In addition, a retrospective, two-day feeding questionnaire was completed at each visit. This included details of how much formula was prepared, how much remained in the bottle after each feed, and details of any other foods or liquids given in addition to the study formula. Furthermore, the mothers were instructed to return all unused formula at each visit and the returned amounts were correlated with the amounts that the infant was reported to have taken. A record of the stool pattern over the previous two days was collected in terms of stool frequency, colour and consistency. In addition, the frequency of spitting up and vomiting, and the infant's behaviour with respect to "periods of unrest", were recorded.

The study physicians collected blood specimens from a peripheral vein for all the infants on days 42, 119 and 182, where possible just before the next feed. On each of these occasions, blood was taken for the measurement of haemoglobin, albumin, total protein, blood urea, creatinine, quantitative amino acids, ferritin, C-reactive protein, calcium, sodium, phosphate, potassium, chloride and glucose. In addition, IgE (total and specific for α-lactalbumin, β-lactoglobulin, and total milk proteins) and IgG to hepatitis B surface protein (hepatitis B vaccine is given routinely in South Africa at six, 10 and 14 weeks of age) were assayed to assess immune status. Haemoglobin measurements were done by the local hospital laboratories and all the other assays were performed by the Centre Hospitalier Universitaire Vaudois, Laboratoire Central de Chimie Clinique in Lausanne, Switzerland. All specimens other than for haemoglobin measurement were collected in heparinised tubes, and put on ice until they were centrifuged within one hour of collection. The supernatant plasma was separated and then stored in a refrigerator at -70°C until it was shipped to Switzerland by a recognised international carrier. In addition to its use in the above tests, blood was collected on days 42 and 119 for HIV DNA polymerase chain reaction (PCR) tests, performed by the virology laboratory of the National Centre for Communicable Diseases in Johannesburg and by the virology laboratory of Groote Schuur Hospital in Cape Town. If there was a discrepancy between the two tests, a third test was done and, for further confirmation, one for viral load was done as well. These results were regarded as definitive. All laboratories used in this study were subjected to internal and external quality control systems.

The study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand and the University of Cape Town. All mothers of infants enrolled gave written informed consent.

Due to the objective of the trial to demonstrate the safety of the acidified formulas, the sample size calculation was for a non-inferiority study in HIV-exposed, uninfected infants concerning weight gain. The data collected by Nelson et al[9] was used as a reference for the sample size calculation, with a mean gain in weight of 28 g/day (SD = 5.7) regarded as normal. A tolerance of 3.9 g/day between enrolment and four months of age was accepted as non-inferior. A total number of 84 infants (28 per formulation group) were required to complete the study. On the basis of a previous study,[10] it was initially estimated that a total of 120 infants would be sufficient for the required number of 84 HIV-uninfected infants to complete the study. However, allowing for those infected with HIV and those who did not complete the study, 150 (50 infants per formulation group) were ultimately recruited and randomised into the study.

The above calculation was based on the intention to show non-inferiority in mean weight gain over four months in experimental versus control formulation groups. Non-inferiority was declared if the lower limit of a one-sided 95% confidence interval for the mean weight gain differences between the experimental and the control formulation group was entirely above the non-inferiority margin of -3.9 g/day. The above assumed a one-sided two-sample t-test, with a statistical power of 80% and an estimated standard deviation of 5.7 g/day, based on data from Nelson et al.[9] This power calculation was implemented using Power Analysis and Sample Size software (PASS, version 6.0).

An intention to treat (ITT) analysis, as well as a per protocol (PP) analysis, was planned. The growth parameters were calculated as mean growth velocities between 14 and 119 days of age (i.e. the time period for exclusive formula feeding). The effects of the

different dietary regimens were tested by analysis of variance (ANOVA) procedures controlling for gender. The Z-scores of the anthropometric parameters were calculated on the basis of the 2000 Centers for Disease Control growth charts, and the Z-scores at the last visit (182 days) were compared using analysis of covariance (ANCOVA) correcting for the Z-scores at baseline. All statistical analyses were done using SAS software (version 8). A p-value of less than 0.05 was considered as significant.

Results

Enrolment commenced in September 2001 and the follow up of all infants was completed in December 2002. The baseline characteristics of the 150 infants enrolled can be seen in Table I. Of these, a total of 53 'dropped out' during the course of the study and could not be traced, 25 of whom did so before the visit at six weeks of age. HIV status was available for 125 infants on the basis of at least one PCR test. Of these, 16 were HIV positive (12.8%). Of the 109 infants who were HIV negative, a further 20 were lost to follow up, while five mothers fed their infants other milks or foods before four months of age.

Table I: Demographic and anthropometric measurements at birth

Feeding group		CAF	CAFP	CAFPN
Number of subjects		50	50	50
Sex	Male	23	22	22
	Female	27	28	28
Birth weight (g)		3144 ± 401	3067 ± 364	3152 ± 426
Gestational age (wks)		39.3 ± 1.3	39.3 ± 1.4	39.5 ± 1.3
Vaginal delivery		33 (66%)	33 (66%)	34 (68%)
Antiretroviral treatment		50 (100%)	47 (94%)	48 (96%)

CAF: Chemically acidified formula
CAFP: Chemically acidified formula with prebiotics
CAFPN: Chemically acidified formula with prebiotics and nucleotides

Those who were lost to follow up after six weeks were included in the intention-to-treat analysis, 37 of whom received the chemically acidified formula, 40 the acidified formula with prebiotics, and 32 the acidified formula with prebiotics and nucleotides. A total of 84 infants were eligible for the per-protocol analysis, 29 of whom received the chemically acidified formula, 32 the acidified formula with prebiotics, and 23 the acidified formula with prebiotics and nucleotides. These details are summarised in Figure 1.

The groups were comparable at enrolment with respect to birth data, anthropometric measurements, age and gender (Table I). The amount of formula consumed was not different during the course of the study. In the ITT analysis there were no statistically significant differences in average weight gain (g/day). Table II shows that the CAFP and CAFPN groups were not inferior to the CAF group, since the lower bounds of the 95% confidence interval are higher than the equivalence level of -3.9 g/day. Similar results were seen in the PP analysis, as shown in Table III.

At the last visit (182 days) there were no statistically significant differences in the weight Z-scores between the three formula groups (p = 0.36 by ANCOVA, correcting for baseline Z-scores).

Figure 1: Flow diagram of participants

CAF: Chemically acidified formula
CAFP: Chemically acidified formula with prebiotics
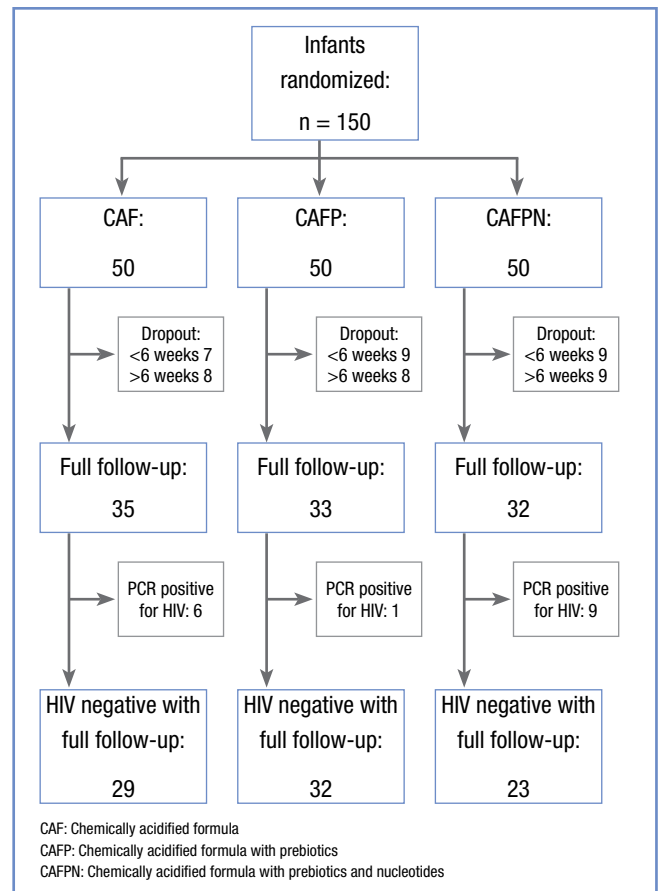CAFPN: Chemically acidified formula with prebiotics and nucleotides

Table II: Comparison of weight gain between the 14-day and 119-day (four month) visit, as analysed on an intention-to-treat basis (Numbers in each group: CAF – 37, CAFP – 40, CAFPN – 32).

	Intention-to-treat analysis (119- to 14-day visits)	
Formulas	Mean (SE)	One-sided 95%CI
CAFP vs CAF	2.34 (1.68)	[-0.45; +∞]
CAFPN vs CAF	0.11 (1.77)	[-2.82; +∞]
CAFPN vs CAFP	-2.40 (1.78)	

Table III: Comparison of weight gain between the 14-day and 119-day (four-month) visit, as analysed in the per-protocol analysis (Numbers in each group: CAF – 29, CAFP – 32, CAFPN – 23).

	Per-protocol analysis (119- to 14-day visits)	
Formulas	Mean (SE)	One-sided 95%CI
CAFP vs CAF	0.50 (1.65)	[-2.24; +∞]
CAFPN vs CAF	-0.64(1.80	[-3.62; +∞]
CAFPN vs CAFP	-1.14 (1.76)	

Increases in length, head circumference and body mass index did not differ between the feeding groups, and at the last visit there was no difference in the length Z-score (p = 0.97), head circumference Z-score (p = 0.12) or weight-for-length Z-score (p = 0.25) between the three formula groups. Figure 2 shows the mean weight Z-scores for the three formula groups during the course of the study. The mean values at baseline were between -0.75 and -0.5, but the

Figure 2: Weight for age Z-scores during the course of the study for the three feeding groups
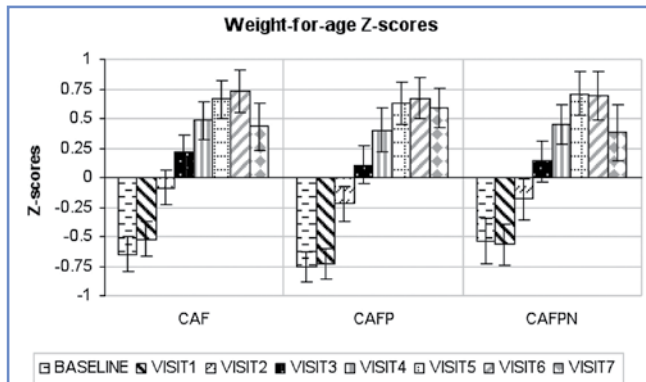
Figure 3: Length for age Z-scores during the course of the study for the three feeding groups
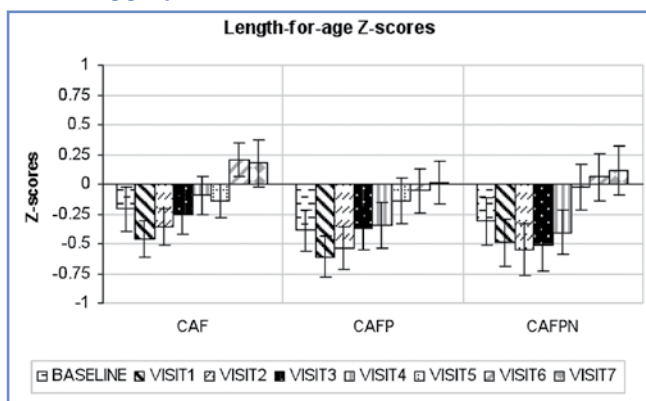
scores increased over time and all groups had positive weight Z-scores by six weeks of age and were between +0.5 and +0.75 by four months of age. Figure 3 shows a similar trend for the length Z-scores. Although the values did not increase as much as those for weight, they were in the positive range at the end of the study for all formula groups.

No statistically significant differences with respect to the frequency, consistency or colour of the stools were observed between the formula groups during the course of the study. The frequency of spitting, vomiting, flatulence and periods of unrest did not differ statistically between the groups. A total of 19 infants required admission to hospital during the course of the study (this figure included those testing HIV positive and/or those who were later withdrawn), three of whom required admission on two occasions. Of these, nine were in the CAF group, seven in the CAFP group and three in the CAFPN group (differences not significant). Nineteen of these admissions were for either lower respiratory tract infections or gastroenteritis, while the other three were for miscellaneous conditions. One infant in the CAFP group died during the course of the study, from gastroenteritis, septicaemia and herbal medicine ingestion on day 39 of the study, prior to an HIV test being done.

No statistically significant differences were noted between the formula groups with respect to haemoglobin or any of the biochemical measures performed, and no significant abnormalities were noted in any of the groups. With respect to the amino acid analyses, the

only differences noted were that aspartate levels were significantly lower in the CAFPN group, while glycine levels were significantly lower in the CAF group. Levels of specific IgE were generally below the detection limit (0.35 kU/l) and therefore were not analysed statistically. Total IgE levels increased with age, but there were no differences in mean levels between the groups. Similarly, hepatitis B antibody titres increased with age, as expected as a result of the immunisation programme, but no differences were noted between the groups.

Discussion

The study corresponded with the time of significant expansion of the government MTCT programme, but the expanded programme developed much more rapidly in the urban centres. As a result, many pregnant women were coming to the urban centres for the benefits of the MTCT programme, giving local addresses and, in a number of cases, enrolling their infants in the study but returning to their rural homes within a few weeks. Thus, although more infants than originally expected failed to complete the study, this was not thought to be related to any problems experienced with the study formulas. However, although there is no reason to suspect that those who were lost to follow up and those who completed the study differed in any way, this is a potential pitfall in the interpretation of the results. There were no differences in the numbers of infants who were lost to follow between the three feeding groups, and the smaller number of HIV-uninfected infants who completed the study in the CAFPN group was mainly as a result of a trend (not statistically significant) towards a larger number of infants becoming HIV infected in that group – an event unrelated to the study formulas.

Both the ITT and the PP analysis showed no differences with respect to growth, and the formulas containing prebiotics with or without nucleotides were not inferior to those without probiotics or nucleotides. This was expected, as neither prebiotics nor nucleotides have previously been shown to enhance growth. What was noteworthy was that the mean Z-scores were below the means of the NCHS norms at the time of enrolment in the study, but that they increased during the course of the study, and the mean weight Z-scores were greater than 0.5 for weight by four months of age and the length Z-scores had become positive by the end of the study. In a previous birth cohort study in Johannesburg during the early 1990s, improvements in weight and length Z-scores were noted in the first six months of life.[11] However, in that study the Z-score was 0.02 for weight and -0.54 for length at six months of age – substantially lower than the scores seen in this study. It is likely that the free supply of milk for this group of infants from poor socio-economic circumstances, the careful and continuous monitoring of their growth and the fact that infants who developed serious illness generally did not complete the study were all factors responsible for the greater gains in weight and length seen in this study.

From a study on premature infants, conducted by Ballabriga et al[12] several decades ago and utilising chemically acidified formulas, it was concluded on safety grounds that only L(+) lactic acid

should be used for the chemical acidification of infant formulas, since the infants who received the D form developed a significant metabolic acidosis. Unlike the L form, which is rapidly metabolised, the D form may accumulate in the body, resulting in a significant acidosis.[13] Since then, the literature on acidified infant formulas has concentrated on formulas that are biologically acidified with lactic acid-producing bacteria during the production process. This is one of the first studies on normal-term infants utilising chemically acidified formulas. Growth and tolerance of the formula were equivalent to the findings of a previous study in which we utilised a standard, non-acidified formula and one that was biologically acidified.[10] Thus, the chemical acidification of a starter infant formula appears to be a realistic alternative to biological acidification and may have benefits with respect to cost and consistency, as discussed previously. Although the acid base status of the infants in this study was not measured systematically, some random venous blood gases were performed and all were normal. The effects of both biologically and chemically acidified formulas on the acid base balance were addressed systematically in a separate study conducted by our group and shown to be safe.[14]

With respect to the differences seen in the amino acid profiles, it should be noted that many parameters were tested and no correction for multiple testing, such as the Bonferroni test, was done. It is probable that these differences were chance findings and that they have no clinical significance.

A number of studies have shown beneficial effects of both prebiotics and nucleotides when added to infant formulas. Prebiotics have been shown to alter the balance of the gut microbiota, with potentially protective effects against diarrhoea and other infective illnesses,[15,16,17] while a recent study showed a reduction in atopic dermatitis during the first six months of life in the group receiving a formula with prebiotics.[18] Nucleotide supplementation has been shown to increase the antibody response to certain immunisations given in infancy, and to reduce episodes of diarrhoea.[6,7,19] Our study was unable to show any of these effects for either the prebiotics or nucleotides utilised in this study. However, since our study was powered to assess growth and not any of these end points, it was probably underpowered to show any such benefits.

With respect to the safety of the formulas, we could find no evidence to suggest any concerns regarding chemical acidification or the addition of prebiotics and/or nucleotides.

Conclusions

All three chemically acidified formulas used in this study were tolerated well. No differences in growth parameters were seen with the addition of prebiotics and nucleotides, and all three groups showed satisfactory growth rates compared with those we have studied on standard formulas.[10,14] Chemical acidification of formulas for healthy term infants did not give rise to any safety concerns in this study and, given the potential cost benefits, such formulas deserve further study.

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