Shao J. Zhou, Thomas Sullivan, Robert A. Gibson, Bo Loennerdal, Colin G. Prosser, Dianne J. Lowry, and Maria Makrides

Nutritional adequacy of goat milk infant formulas for term infants: a double-blind randomised controlled trial

The British Journal of Nutrition, 2014; 111(9):1641-1651

Originally Published at:
http://dx.doi.org/10.1017/S0007114513004212

Title: Nutritional adequacy of goat milk infant formula for term infants: a double-blind randomised controlled trial

Shao J Zhou[1,2,5], Thomas Sullivan[4], Robert A Gibson [5], Bo Lönnerdal[6], Colin G Prosser[7], Dianne J Lowry[7], Maria Makrides[1,2,3]

Author affiliation:

[1] Women's & Children's Health Research Institute, 72 King William Road, North Adelaide, SA 5006, Australia

[2] Department of Paediatrics & Child Health, Flinders Medical Centre, Bedford Park SA 5042, Australia

[3] School of Paediatrics & Reproductive Health, University of Adelaide, Adelaide, SA 5005, Australia

[4] Data Management and Analysis Centre, Discipline of Public Health, University of Adelaide, Adelaide, SA 5005, Australia

[5] School of Agriculture, Food & Wine, University of Adelaide, Waite Campus, Waite Road, Urrbrae, SA 5064, Australia

[6] University of California, Davis, USA

[7] Dairy Goat Co-operative (N.Z.) Ltd, Hamilton, New Zealand

Corresponding author:

Prof Maria Makrides

Women's & Children's Health Research Institute

72 King William Road, North Adelaide, SA 5006, Australia

Telephone: +618 8161 6067

Facsimile: +618 8239 0267

E-mail: maria.makrides@health.sa.gov.au

Reprints are not available from the authors

Running title: Infant Goat formula, growth & nutrition

Keywords: infant, growth, breastfeeding, formula, goat milk

Clinical trial registry: Australian New Zealand Clinical Trials Registry (ACTRN12608000047392).

Abstract

The safety and nutritional adequacy of Goat milk infant formulae has been questioned. The primary objective of this study was to compare growth and nutritional status of infants fed goat milk infant formula with a typical whey based cow milk infant formula. The secondary aim was to examine a range of health and allergy-related symptoms. A double blind, randomised controlled trial with 200 formula fed term infants randomly assigned to receive either goat or cow milk formula from 2 weeks until at least 4 months of age was conducted. A cohort of 101 breastfed infants was included for comparison. Weight, length and head circumference were measured at 2 weeks, 1, 2, 3, 4, 6 and 12 months of age. Nutritional status was assessed from serum albumin, urea, creatinine, haemoglobin, ferritin, folate and plasma amino acids at 4 months. Z-scores for weight, length, head circumference and weight for length were not different between the two formula groups. There were differences between formula groups in some amino acids and blood biomarkers, but the mean values for biomarkers were within the normal reference range. There were no differences in occurrence of serious adverse events, general health, incidence of dermatitis or medically diagnosed food allergy. The incidence of parental reports blood stained stools was higher in the goat milk formula group, although this was a secondary outcome and its importance is uncertain. Goat milk formula provided growth and nutritional outcomes in infants that did not differ from a standard whey based cow milk formula.

Appropriate nutrition during infancy is important not only for normal growth and development of the infant, but also for long term health outcomes. Breast feeding is recommended for delivering these short and long-term outcomes [1]. Infant formulas are used to supplement breast milk when breast milk is not sufficient or breastfeeding is not possible. Cow milk infant formula is widely accepted as the first-line choice for healthy formula-fed infants. These are typically based on cow milk proteins from skim milk and have extra whey proteins added to improve the profile of essential and semi-essential amino acids [2, 3].

There is also consumer demand for goat milk infant formula as evidenced by widespread reports of the use of raw goat milk and homemade formula for infants [4-7]. Goat infant formulae are manufactured in several countries. Compositional analysis of an infant formula made from goat milk without added whey proteins suggests that the amino acid profile [8] is compatible with international standards for infant formula [9, 10]. This type of goat milk formula was also shown in animal studies to have similar digestibility and absorption of amino acids compared with a cow infant formula with added whey [11]. Thus, it was expected that the amino acid delivery to infants would be similar between the two formulae but this has never been tested.

In addition to meeting compositional criteria it is important to establish the suitability and nutritional adequacy of infant formula containing new sources of proteins through clinical trials [9, 12]. While goat milk has high quality proteins and fats and has a history of use for human nutrition in many cultures [13-15], there has been only one previous randomised controlled trial (RCT) of infants fed goat milk infant formula [16]. This study showed that growth of 30 infants fed goat milk infant formula was similar to 32 infants fed a whey based cow milk infant formula [16]. However, that study was insufficient for assessing the safety and nutritional adequacy of the goat milk formula because it was underpowered and lacked blood biochemical data [17].

The primary aim of the present study was to compare growth and nutritional status of infants fed formulas either based on goat milk or cow milk in a well powered RCT. The secondary aim was to examine a range of health and allergy-related symptoms, including incidence and severity of dermatitis.

Materials and methods

Participants

The study population included two cohorts of infants who were either fed infant formula or were breastfed at the time of recruitment. Infants were eligible for inclusion in the study if the following inclusion criteria were met: 1) a healthy term infant with gestation of 37-42 weeks and birth weight $\geq$ 2.5 kg and $\leq$ 4.75 kg; 2) aged up to 2 weeks; 3) mother was exclusively feeding infant formula within 2 weeks of birth (for formula cohort) or planned to exclusively breastfeed for at least 4 months (for the breastfed cohort). Infants were excluded if they were from multiple births or had severe congenital or metabolic disease likely to affect infant feeding or infant growth. Infants who were exclusively formula fed or breastfed were identified and referred by midwives in the postnatal wards at one of three tertiary hospitals, the Women's & Children's Hospital, the Flinders Medical Centre or the Lyell McEwin Hospital in Adelaide, Australia. The study was approved by the relevant Human Research Ethics Committees at all three study centres. Written informed consent was obtained from all participating families. The trial was registered with Australian New Zealand Clinical Trials Registry (ACTRN12608000047392).

The nutrition composition of the study formulas

The goat infant formula (GIF) was manufactured by Dairy Goat Co-operative (N.Z.) Ltd using whole goat milk without added whey proteins (final whey to casein ratio of approximately 20:80) and a blend of approximately 60% milk fat and 40% vegetable oils. The control cow infant formula (CIF) consisted of cow skim milk and whey proteins (final whey to casein ratio of approximately 60:40) and vegetable oils as the source of fat and supplied by Nutricia (Auckland, New Zealand). The protein to energy ratio of the both study formula was at the lower limit specified by CODEX [10] and similar to the low protein formula that is suggested to provide a more desired weight gain in infants [18]. The nutritional composition of both formulas is listed in Table 1.

Study allocation and blinding

Eligible formula fed infants were randomly assigned to GIF or CIF. Treatment allocation was through a web-based randomization service according to a computer generated randomization schedule, which was prepared by an independent statistician. Stratification was by sex and study centre and used variable block sizes of 4 and 8 in

equal proportions. The formulas were labeled in four different colors, two of them corresponding to GIF and the other two corresponding to CIF. Cans of both formulas were otherwise identical in appearance to maintain the blind. This ensured that neither the parents nor the research staff were aware if the formula allocated was GIF or CIF. The blinding index was used to assess the success of blinding [19].

Study intervention

Parents and caregivers of formula fed infants were asked to feed their infants the allocated study formula from enrolment to at least four months of age and thereafter with other complementary foods up to 12 months of age. Study formulas were supplied free of charge until 12 months of age. For breastfeeding infants, mothers were encouraged to continue exclusive breastfeeding for around four to six months of age in line with current recommendations. Support for breastfeeding was provided by a qualified lactation consultant to mothers free of charge if needed. The timing of introduction of solids around 4 and 6 months was at the discretion of the families for both the formula fed and the breast fed infants.

Outcome assessments

The primary outcomes were infant weight, length and head circumference, measured at enrolment, 2 weeks and 1, 2, 3, 4, 6 and 12 months. All anthropometric growth data were converted to z-scores using WHO Child Growth Standards (http://www.who.int/childgrowth/en/). Secondary outcomes included nutritional status, general health, tolerance to formula and allergy symptoms.

A small non-fasting blood sample (3-5 mL) was collected to assess blood biomarkers, including haemoglobin, packed cell volume (PCV) and serum creatinine, urea, albumin, ferritin, folate and plasma amino acids, at 4 months of age as indicators of general nutritional status. Iron deficiency anaemia was defined as haemoglobin < 100 g/L & ferritin < 20 µg/L based on the diagnostic criteria of the test laboratory. Hemoglobin was measured spectrophotometrically by using a Cell Dyn 4000 analyzer (Abbott Laboratories, Santa Clara, CA), which has a coefficient of variation (CV) of <2%. Albumin, urea and ferritin were measured by Cobas/Hitachi Cobas C System, Cobas 6000 automated analyser (Roche Diagnostics, Indianapolis IN). Albumin was determined spectrophotometrically by an end-point BCG Dye-binding method. Urea was measured spectrophotometrically by an enzymatic method. The test method for

ferritin was particle enhanced immunoturbidmetry. The measurement of albumin and urea have CVs of <3% and ferritin has a CV <4%. Serum folate was analysed by ARCHITECT *i* optical system (Abbott) using the Chemiluminescent Microparticle Immunoassay (CMIA) Technology and has < 4% CV. Amino acids were measured on Hitachi L-8900 Amino Acid Analyser. Plasma samples (200 uL) were acidified with 50 ul sulfosalicyclic acid to precipitate intact protein prior to analysis. The supernatant was mixed with lithium-diluent spiked with AE-Cys. The L-8900 Hitachi analyzer utilizes a lithium citrate buffer system and ion- exchange (Hitachicolumn) chromatography to separate amino acids followed by a "post-column" ninhydrin reaction detection system.

At each growth assessment time point, parents/care givers were asked through a structured interview whether their infant had experienced any health problems including respiratory illness, gastro-intestinal illness, reflux, eye infection, ear, nose and throat conditions, fever, urinary tract infection and thrush. Serious adverse events, defined as death or hospital admission > 24 hour during the 12 months study period, were also recorded.

At the same time of growth assessments, incidence of dermatitis and its severity was assessed by trained research staff using SCORAD [20]. Food allergy was diagnosed by medical practitioners. Parents/care givers were also asked whether their infants had have symptoms related to food allergy and/or gastrointestinal function including hives, swelling of the face or body, wheeze/stridor, vomiting, loose watery stools, blood stained stools and itchy rash.

Parents/care givers were asked to assess stool frequency, consistency and effort as indicators of tolerance to formula using the Bristol Stool Scale [21] as a guide. Sleeping patterns including length of each sleep, total number of sleeps during the day, and the length of time taken to settle for sleep during the day, in the evening or at night were also assessed by parental report based on the Sleep and Settle Questionnaire [22].

Other assessments

Demographic and baseline characteristics, including infant sex, weight and length at birth, age at enrolment, anthropometric measurements at enrolment, maternal age, BMI, parity, and history of smoking and drug and alcohol use during pregnancy were recorded at trial entry.

160

161 Sample size and power calculation

162 Sample size calculations estimated that 64 infants per group were required to detect a
163 0.5 SD difference (80% power with α=0.05) in weight [12]. We aimed to enrol 100
164 infants per feeding group and 100 breastfed infants to provide reference data from a
165 breastfed group. This sample size was also sufficient to detect a clinically important
166 difference of 0.11 g/L (SD of 0.26g/L) in serum albumin, an indicator of protein
167 adequacy, with 80% power (α=0.05).

168

169 Statistical analysis

170 All analyses were performed using SAS® Software version 9.2 or later (SAS Institute
171 Inc., Cary, NC, USA). Blinded treatment codes were included in the database and
172 analyses of the primary and secondary outcomes were performed blinded to treatment
173 group. All analyses were performed using both intention-to-treat and per-protocol
174 approaches, with infants who did not complete the trial or who had any non-study
175 formula, liquids or solids for more than 12 days between 2 weeks and 4 months of age
176 were excluded from the per-protocol analysis. As the two analysis approaches
177 produced similar results, only the primary intention-to-treat analyses are reported
178 here.

179 In order to minimize bias in the estimation of treatment effects due to missing data,
180 multiple imputation was used to create 50 complete datasets for analysis. The
181 parametric regression method was used to impute continuous variables and the
182 logistic regression method was used for binary variables. In addition to the primary
183 imputed analysis, sensitivity analyses were performed on the original data and on
184 imputed data created using different seeds and using different imputation models. All
185 approaches produced similar results, thus only the results of the primary imputed
186 analysis are presented.

187 Continuous outcomes measured at multiple assessments, including the primary
188 anthropometric outcomes, were compared between formula and breastfeeding groups
189 over time using linear mixed effects models. Fixed effects for group, time and the
190 interaction between group and time were included in the models, while dependence
191 was accounted for by allowing for correlated residuals within a child. Independent of
192 the statistical significance of the interaction term, differences between groups were

reported separately at each time point, with the effects of treatment group expressed as mean differences. Continuous outcomes measured at a single time point were compared between groups using linear regression models, with the effects of group expressed as mean differences. Binary outcomes were analyzed using log binomial regression models, with the effects of group expressed as relative risks. Rare binary outcomes were analyzed using Fisher exact tests. Both unadjusted and adjusted analyses were performed, with conclusions on group differences being based on the adjusted analyses. For the primary growth outcomes, comparisons of the two randomised groups were adjusted for centre, while comparisons involving the breastfed reference group were adjusted for maternal education and the relevant anthropometric z-score at birth. All secondary outcomes were adjusted for the stratification variables centre and sex for comparisons of the randomised groups and maternal education and birth weight for comparisons involving the breastfed reference group. Due to imbalances in maternal smoking during pregnancy between the randomised groups, sensitivity analyses of the primary growth outcomes adjusting for centre and maternal smoking during pregnancy were also performed. All tests were two tailed with a significance level of $P \leq 0.05$.

Results

Participants were recruited between April 2008 and April 2009 from three tertiary hospitals in Adelaide. Of the 1180 families who were approached to participate in the study, 768 were eligible and 301 (39%) consented. Two hundred infants were formula fed and 101 were breastfed. See the participant flow chart for more details (Figure 1).

Maternal characteristics as well as infant anthropometrics at birth and at study entry are presented in Table 2. The mean age of infants at study entry was 6.2 ± 3.7 (standard deviation) days and 46% were male. The baseline characteristics of the participants were comparable between the two formula groups, with the exception that the percentage of mothers who smoked during pregnancy was higher in the GIF group (45%) compared with the CIF group (34%). Compared with formula fed infants, the reference group of breastfed infants had a higher mean birth weight ($p=0.001$), lower maternal pre-pregnancy BMI ($p < 0.0001$), lower percentage of maternal smoking ($p < 0.0001$) during pregnancy and higher percentage of parents who completed higher education ($p< 0.0001$). The percentage of mothers who did not know their baby's treatment group was similar between the groups (32% in the GIF group and 34% in

the CIF group). The blindness index, which indicates the percentage of mothers who guessed their treatment group correctly above chance, was 3.8% for the GIF group compared with 2.7% for the CIF group.

The median (inter-quartile (IQ) range) daily intake of study formula ranged from 698 ml (570 – 825 ml) in the first 2 weeks to 1000 ml (855 – 1190 ml) at 4 and 6 months. Seventy-five percent (76/101) of the breast fed infants, 73% (74/101) of infants in the GIF and 60% (59/99) in the CIF group were compliant with the definition of exclusive formula feeding or breast feeding [23] from enrolment to 4 months of age. The level of compliance in the GIF was significantly different to CIF (p=0.02), but not significantly different to the breast fed reference group (p=0.37).

Growth

There were no differences between the two formula groups over the 12 month study period in the adjusted intention to treat analyses of weight (Figure 2a), length (Figure 2b**),** head circumference (Figure 2c**)** and weight-for-length (Figure 2d) z-scores, with or without adjustment for baseline difference in maternal smoking. Also, gains in weight, length or head circumference from registration to 4 or 6 months did not differ between the two formula groups (data not shown).

In comparison with breastfed infants, infants in the GIF group had higher weight z-scores at 3, 4 and 6 months (mean difference 0.22, p=0.04; 0.30, p=0.005 and 0.33, p=0.003) while infants in the CIF group had higher weight z-scores from 2 to 12 months of age (mean differences 0.22, p=0.04; 0.28, p=0.01; 0.39, p=0.001; 0.38, p=0.001 and 0.36, p=0.001). Infants in the GIF group had lower length z-scores at 2 weeks and 1 month of age compared with breastfed infants (mean difference -0.33, p=0.003 and -0.37, p=0.001) whereas infants in the CIF group had higher length z-scores at 4, 6 and 12 months of age (mean difference 0.25, p=0.03; 0.35, p=0.002 and 0.25, p=0.03). While head circumference z-scores did not differ between the GIF group and breastfed infants, infants in the CIF group had higher z-scores at 2 and 6 months of age compared with breastfed infants (0.24, p=0.04 and 0.3, p=0.01). Infants in the GIF group had higher weight-for-length z-scores compared with breast fed infants at 1 month only (mean difference 0.40, p=0.004), while weight-for-length z-scores were higher at 1 and 2 months in the CIF group (mean difference 0.46, p=0.001 and 0.39, p=0.006). There were no statistically significant differences between formula and breast fed groups at any other times.

Biomarkers of nutritional status

There were no differences in serum albumin, haemoglobin, PCV and ferritin between the two formula fed groups. No infants in either formula group had iron deficiency anaemia (defined as haemoglobin <100 g/L & ferritin < 20 µg/L). Infants in the GIF group had lower mean serum urea, creatinine and folate concentrations compared with infants in the CIF group (Table 3). Compared with breastfed infants, formula fed infants had higher mean serum urea concentrations, infants in the GIF group had lower mean serum folate concentration and, infants in the CIF group had higher mean folate concentrations (Table 3). The mean serum folate concentrations for all 3 groups of infants were within the normal reference range for infants of this age [24].

Concentrations of essential and semi-essential amino acids in plasma of infants are presented in Figure 3. Valine and phenylalanine were higher and isoleucine and threonine were lower in plasma of infants fed GIF compared with CIF. The mean difference (95% confidence interval (CI)) for valine was 37 (25, 50) µg/L, phenylalanine was 5 (0, 10) µg/L, isoleucine -9 (-16, -3) µg/L and threonine -32 (-45, -18) µg/L. All other essential and semi-essential amino acids in plasma of formula fed infants did not significantly differ between groups.

Compared with breast fed infants, infants fed GIF had significantly higher concentrations of lysine, methionine, phenylalanine, threonine and valine. Mean differences (95% CI) were 15 (1, 29) µg/L, 6 (4, 9) µg/L, 13 (7, 18) µg/L, 13 (7, 18) µg/L, 19 (4, 34) µg/L and 66 (52, 79) µg/L, respectively. Isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine were all higher in plasma of infants fed CIF compared with breast fed infants. Mean differences (95% CI) were 13 (7, 20) µg/L, 11 (2, 21) µg/L, 19 (6, 33) µg/L, 6 (3, 8) µg/L, 8 (2, 13) µg/L, 51 (37, 66) µg/L and 29 (15, 44) µg/L, respectively. No amino acids were lower in either formula group compared with breast fed infants.

General health and allergy-related outcomes

There were no differences in the risk between the two formula groups of an adverse health condition, including respiratory, gastro-intestinal illness, reflux, eye infection, ear, nose and throat conditions, fever, urinary tract infection and thrush. There were also no differences in the risk between the formula groups and the breastfed reference

group for the above health conditions, with the exception that more infants had oral thrush in the CIF group compared with the breastfed reference group (9/86 vs. 2/99, p= 0.02) during the 12 month study period. The proportion of infants who had any serious adverse events during the 12 month study period was similar between the GIF, CIF and breastfed reference groups: 15/101 (14.9%), 12/99 (12.1%) and 9/101 (8.9%), respectively (p=0.43). The most common serious adverse events were bronchiolitis and other respiratory infections. No infants died.

The proportions of infants with medically diagnosed food allergy (GIF 2/92 vs. CIF 1/89 vs. breast fed 5/99) or dermatitis assessed using SCORAD (GIF 13/91 vs. CIF 20/86 vs. BF 21/99) did not differ between groups. The mean SCORAD score of infants with dermatitis was 9.9 $\pm$ 6.7 for GIF, 11.9 $\pm$ 7.1 for CIF and 11.1 $\pm$ 6.3 for breast fed groups (mean $\pm$ SD).

There was no difference between the formula groups in the proportion of infants with parental reported symptoms that related to allergy and/or gastrointestinal function, except for parentally reported blood stained stools (Table 4). Compared with breastfed infants, infants in the GIF group had a higher risk of blood stained stools while infants in the CIF group had a higher risk of wheeze (Table 4). The proportions of infants with hives (GIF 5/89 vs CIF 5/86 vs BF 6/99), swelling of the face (GIF 6/89 vs. 6/86 vs. BF 5/99) did not differ between all groups in simple unadjusted analyses.

Formula tolerance

The mean number of stool motions per day in infants from the GIF group at 2 weeks, 1, 2 and 3 months of age were 2.5 $\pm$ 1.6, 2.0 $\pm$ 1.3, 1.6 $\pm$ 1.0 and 1.6 $\pm$ 0.9 (mean $\pm$ SD), respectively. These were not different from the stool frequency of infants in the CIF group, which were 2.5 $\pm$ 1.4, 2.0 $\pm$ 1.4, 1.5 $\pm$ 0.9 and 1.6 $\pm$ 1.3 at 2 weeks, 1, 2 and 3 months, respectively. However, stool frequency in both formula groups were significantly lower ($p<0.001$) than the breast fed group (6.3 $\pm$ 3.3, 5.0 $\pm$ 2.3, 3.0 $\pm$ 2.2 and 2.4 $\pm$ 1.8 at 2 weeks, 1, 2 and 3 months, respectively). Compared with the CIF group infants in the GIF had lower mean stool consistency scores at 2 weeks (GIF 4.69 $\pm$ 1.44 vs. CIF 5.46 $\pm$ 0.96, $p < 0.0001$) and 1 month (GIF 4.95 $\pm$ 1.35 vs. CIF 5.35 $\pm$ 1.19, $p = 0.01$). No differences in the stool consistency score were observed at other assessment time points.

There were no differences in the mean length of each sleep or the total number of sleeps between the two formula groups, with the exception that infants in the GIF group had a shorter mean length of each sleep in the evening (GIF 103 ± 63 vs. CIF 127 ± 65 minutes, p=0.007) and a longer mean length of each sleep at night (GIF 317 ± 96 vs. CIF 288 ± 102 minutes, p=0.03) at the 2 month assessment. The mean length of time taken to settle for sleep during the day, in the evening or at night also did not differ between GIF and CIF groups. In comparison with breastfed infants, there were some differences in sleeping patterns between the formula fed and the breastfed infants, but the differences were inconsistent (data not shown).

Discussion

This study is the first to rigorously evaluate in healthy term infants the effect of feeding of goat infant formula to 12 months on growth, nutritional status, oral tolerance and a wide range of health and allergy related outcomes in a well conducted RCT involving a control group fed cow milk infant formula and a reference group of breastfed infants. We could detect no difference in z-scores for infant weight, length, head circumference and weight-for-length up to 12 months between the two formula groups. The same overall treatment effects were observed from intention to treat or per-protocol analysis that excluded data from infants who received any non-study formula, liquids or solids for more than 12 days before the four months of age. This suggests it is unlikely that the use of non-study foods by some infants within the first four months had a significant impact on the outcomes of the study. We did detect some differences in weight and weight-for-length z-scores for both formula fed groups compared with breastfed infants, consistent with other studies comparing growth of formula and breastfed infants [25-27]. Interestingly while the differences in weight or weight for length z-scores persisted at 12 months between breastfed infants and infants fed cow milk formula in our study, consistent with the other cow milk based formula studies [25-27] , there was no differences between infants fed goat milk formula and breastfed infants. Our study used the same formula with a lower protein content (2 g/100 kcal and 2.1 g/100 Kcal for goat and cow milk formula, respectively) through to 12 months rather than switching to a follow-on formula with higher protein content from 6 months as occurred in the other formula studies [25-27]. This may partly explain the difference observed between our study and the other formula studies mentioned above as it has been shown that weight for length z-score at 24 months of

361 infants fed low protein formula was not different to breast fed infants while infants
362 fed high protein formula (2.9 g/ 100 kcal) had higher z-score.
363 There were minor differences in the blood biomarkers between the formula fed
364 groups, which likely reflected differences in the composition of the two formulae.
365 For instance, the cow infant formula contained added folate close to the recommended
366 maximum, compared with the goat milk formula that had an amount in the mid-range
367 of the recommendations [9, 10]. Nevertheless, concentrations of blood biomarkers
368 measured at four months were within the normal reference range for infants of this
369 age [24].
370 Whey proteins are often added to formula to help improve protein quality and
371 availability of essential and semi-essential amino acids [28, 29]. Infant formula made
372 from goat milk without added whey proteins was shown to have sufficient quantities
373 of all the essential and semi-essential amino acids [8] and similar digestion and
374 absorption of the amino acids in an animal model compared with a whey based cow
375 infant formula [11]. The present study shows some differences in plasma amino acids
376 profile between the formula groups as well as in comparison with the breastfed
377 infants, but there were large inter-individual variations. Although the differences were
378 statistically significant, they are unlikely to be clinically important as the mean
379 plasma amino acid concentration of infants in both formula groups are comparable
380 with those reported in other studies [30, 31].
381 This study is the first to record a wide range of outcomes related to general health,
382 gastrointestinal function and allergy when infants were exposed to goat infant formula
383 using a combination of objective clinical assessments and subjective parental reports.
384 There were no differences in objective assessments of allergy related outcomes
385 including dermatitis and medically diagnosed food allergy.
386 The only statistically significant finding between the formula groups was a greater
387 number of parental reports of blood stained stools in infants fed goat compared with
388 cow infant formula. We are unsure about the significance of this finding. Firstly, the
389 number of reports of blood stained stools were low overall and secondly, there was no
390 indication of other gastrointestinal disorders, differences in stool characteristics,
391 crying and sleeping patterns, general health or other allergy-related symptoms.
392 Furthermore, none of the infants in the study had iron deficiency anaemia which
393 would indicate no significant blood loss over time. Finally, the outcomes related to
394 allergy and gastrointestinal function were secondary outcomes, which the study did

not have adequate power to rigorously assess, and thus they need to be interpreted with caution as it is possible that this may due to chance. A much larger, adequately powered RCT with objective assessment of clinical outcomes and biomarkers of allergy is needed to rigorously evaluate the effects of goat milk infant formula on allergy and gastrointestinal function.

In conclusion, growth and blood biomarkers of nutritional status of infants fed a whole goat milk based infant formula did not differ from infants fed standard cow infant formula with added whey. The lack of significant difference between the formula groups for an extensive range of health related outcomes and for the occurrence of serious adverse events support the safety of the goat milk for infant formula.

Acknowledgements

We thank the families who participated, the medical, nursing and research staff in each participating centre, the staff of the Child Nutrition Research Centre, the staff of the Data Management and Analysis Centre, University of Adelaide and University of California, Davis, USA. MM and RAG were supported by a National Health & Medical Research Council Senior Research Fellowship (ID: 565000 for MM and ID: 519324 for RAG). Infrastructure support was provided by the Women's and Children's Health Research Institute, The University of Adelaide, Women's and Children's Hospital Adelaide, Flinders Medical Centre Adelaide, Lyell McEwin Hospital Adelaide.

Financial support

Dairy Goat Co-operative (N.Z.) Ltd, New Zealand provided the funding to conduct the study. The funder contributed to the study design, interpretation of findings and the preparation of the manuscript. Data collection, management and analysis were conducted independently of the funder.

Conflicts of interest

Makrides serves on scientific advisory boards for Nestle, Fonterra and Nutricia. Gibson serves on scientific advisory board for Fonterra. Associated honoraria for Makrides and Gibson are paid to their institutions to support conference travel and continuing education for post-graduate students and early career researchers. Prosser & Lowry work for the Dairy Goat Co-operative (N.Z.) Ltd that manufactured the goat milk formula used in the study. No other conflicts of interest were reported.

Authors' contributions:

Designed research: Makrides, Zhou, Gibson, Sullivan, Prosser, Lowry

Conducted research: Makrides, Zhou, Gibson, Lonnerdal

Analyzed data or performed statistical analysis: Sullivan, Zhou, Makrides.

Wrote paper: Zhou drafted the manuscript with contributions from all authors. All authors reviewed and approved the manuscript submitted.

Primary responsibility for final content: Makrides, Zhou.

FIGURE 1 Participant flow through study

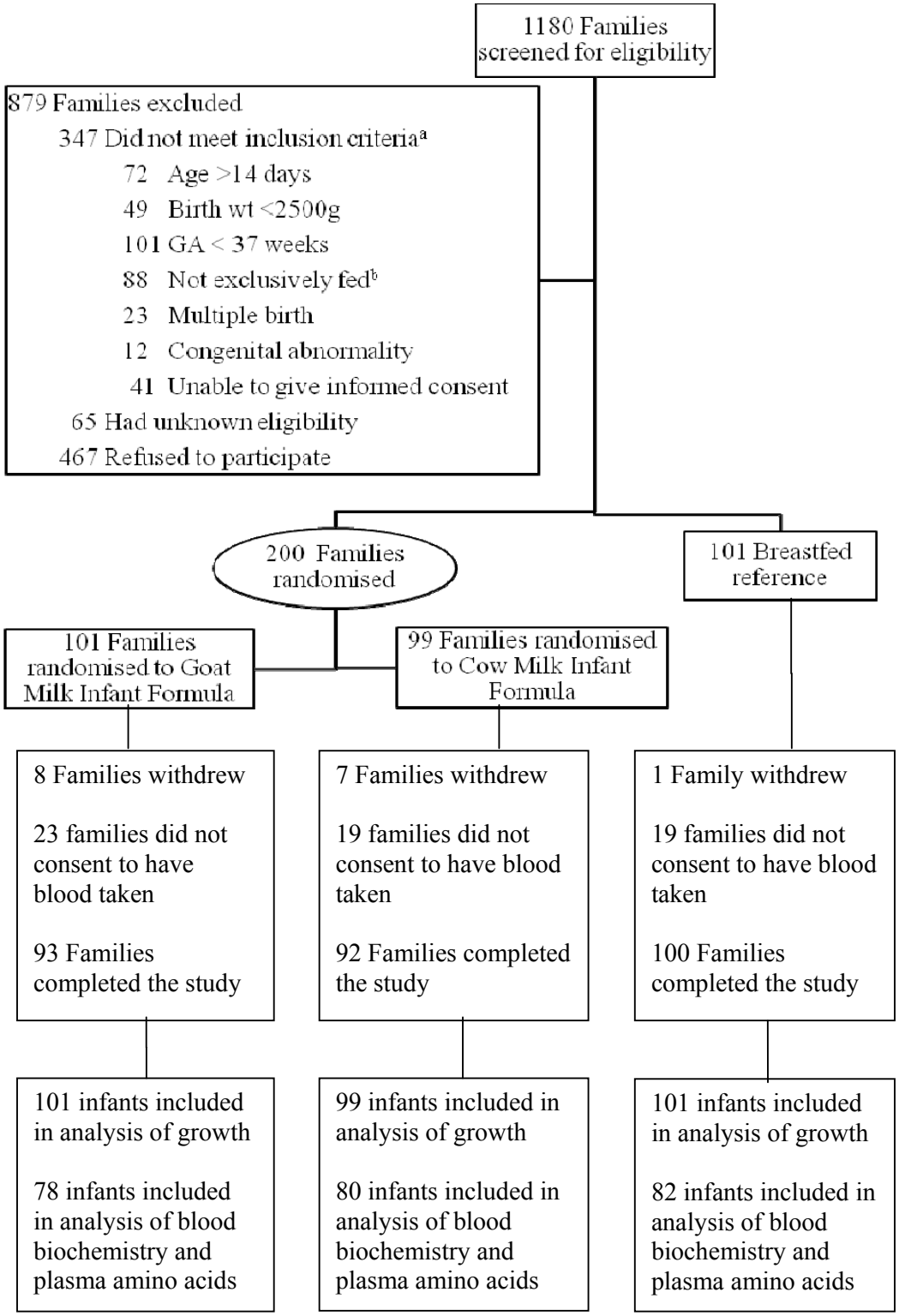

1180 Families screened for eligibility
879 Families excluded
347 Did not meet inclusion criteria[a]
72 Age >14 days
49 Birth wt <2500g
101 GA < 37 weeks
88 Not exclusively fed[b]
23 Multiple birth
12 Congenital abnormality
41 Unable to give informed consent
65 Had unknown eligibility
467 Refused to participate
200 Families randomised
101 Breastfed reference
101 Families randomised to Goat Milk Infant Formula
99 Families randomised to Cow Milk Infant Formula
8 Families withdrew
23 families did not consent to have blood taken
93 Families completed the study
7 Families withdrew
19 families did not consent to have blood taken
92 Families completed the study
1 Family withdrew
19 families did not consent to have blood taken
100 Families completed the study
101 infants included in analysis of growth
78 infants included in analysis of blood biochemistry and plasma amino acids
99 infants included in analysis of growth
80 infants included in analysis of blood biochemistry and plasma amino acids
101 infants included in analysis of growth
82 infants included in analysis of blood biochemistry and plasma amino acids

Table 1. Nutritional composition of the two infant formulas used in the study.

		Goat milk formula	Cow milk formula	Mature human milk[1]
Nutrient	Unit	Per 100 mL	Per 100 mL	Per 100 g
Energy[2]	kcal	65.6	64.8	70
	kJ	274.0	271.0	291
		Per 100 kcal	Per 100 kcal	Per 100 g
Protein	g	2.0	2.1	1.0
Fat	g	5.3	5.2	4.4
Saturated fat	g	2.0	2.0	-
Unsaturated fat	g	3.3	3.2	-
Linoleic acid ώ6	g	0.6	0.9	-
α-Linolenic acid ώ3	g	0.1	0.1	-
Carbohydrate	g	11.0	11.0	6.9
Vitamins				
Vitamin A (RE)	µg	141.0	87.0	61
Vitamin D_3	µg	1.8	2.1	0.1
Vitamin E (TE)	mg	2.6	1.1	0.08
Vitamin K_1	µg	12.0	8.8	-
Vitamin C	mg	20.0	12.0	5
Thiamine	µg	118.0	58.0	10
Riboflavin	µg	226.0	250.0	40
Niacin	mg	1.3	0.8	0.18
Vitamin B_6	µg	80.0	65.0	-
Folic acid	µg	12.0	21.0	5.0[3]
Pantothenic acid	mg	0.6	1.2	0.22
Vitamin B_{12}	µg	0.3	0.5	0.05
Biotin	µg	3.8	4.7	-
Minerals				
Calcium	mg	98.0	81.0	32
Phosphorus	mg	73.0	53.0	14
Sodium	mg	31.0	31.0	17
Potassium	mg	133.0	116.0	51
Chloride	mg	116.0	71.0	-
Magnesium	mg	10.0	10.0	3
Iron	mg	1.0	1.3	Trace
Zinc	mg	0.9	0.7	0.2
Iodine	µg	15.0	17.0	-
Copper	µg	76.0	70.0	0.1
Manganese	µg	16.0	12.0	-
Selenium	µg	1.9	3.7	1.8
Inositol	mg	6.8	5.1	-
Choline	mg	27.0	19.0	-
Taurine	mg	8.9	6.6	-
Carnitine	mg	1.2	3.3	-

[1]Reference: Wijesinha-Bettoni, R & Burlingame, B. Chapter 3. Milk and dairy products composition. In: Muehlhoff, E, Bennett, A & McMahon, D eds. Milk and dairy products in

human nutrition. FAO 2013. [2]The energy content was calculated based on 14 g powder added to 100 mL water. [3]Folate

Table 2. Characteristics of participants.

	GIF (n=101)	CIF (n=99)	BF (n=101)	P-value[2] (FF vs. BF)
Maternal characteristics				
Age (y)	27.8 ± 6.6[1]	28.2 ± 5.8	30.7 ± 5.2	0.0002
Race, Caucasian [n (%)]	92 (91)	94 (95)	93 (92)	
Education [n (%)]				<0.0001
Secondary incomplete	30 (30)	36 (36)	10 (10)	
Certificate/diploma or secondary complete	65 (64)	58 (59)	50 (50)	
Degree or higher degree	6 (6)	5 (5)	41 (41)	
BMI (kg/m^2)	26.6 ± 6.3	27.8 ± 7.6	24.6 ± 4.5	0.0007
Smoking in pregnancy [n (%)]	45 (44.6)	34 (34.3)	10 (9.9)	<0.0001
Infant				
Birth characteristics				
Sex, M [n (%)]	48 (47.5)	45 (45.5)	44 (43.6)	0.63
GA at birth (wk)	39.4 ± 1.0	39.3 ± 1.1	39.6 ± 1.0	0.048
Birth weight (g)	3379 ± 466	3407 ± 419	3564 ± 409	0.001
Birth length (cm)	49.5 ± 2.0	49.3 ± 2.1	50.2 ± 2.0	0.003
Birth head circumference (cm)	34.7 ± 1.4	34.6 ± 1.5	35.1 ± 1.2	0.01
Baseline data				
Age at enrolment (d)	6.0 ± 3.6	6.1 ± 3.7	6.5 ± 3.8	0.35
Weight at enrolment (g)	3345 ± 452	3371 ± 423	3491 ± 447	0.01
Length at enrolment (cm)	50.0 ± 2.0	49.9 ± 2.1	50.9 ± 2.0	0.0001
Head circumference at enrolment (cm)	35.0 ± 1.2	35.1 ± 1.4	35.5 ± 1.3	0.009

[1]Mean ± SD (all such values); [2]Continuous and categorical characteristics compared using independent samples t-tests and chi-square tests respectively; GIF: goat milk infant formula; CIF: cow milk infant formula; FF: formula fed; BF: breastfed. GA: gestational age

Table 3. Serum biomarkers at 4 months of age

	GIF (n=78)	CIF (n=80)	BF (n=82)	Adjusted Effect (95% CI) GIF vs. CIF	P	Adjusted Effect (95% CI) GIF vs. BF	P	Adjusted Effect (95% CI) CIF vs. BF	P
Albumin (g/L)	44.6 ± 2.2[1]	44.7 ± 2.5	45.5 ± 2.8	-0.1 (-0.9, 0.7)	0.82	-1.0 (-1.9, 0)	0.04	-0.9 (-1.8, 0.1)	0.07
Creatinine (mmol/L)	17.0 ± 3.2	19.0 ± 3.3	18.5 ±3.4	-2.0 (-3.1, -0.9)	0.0004	-1.0 (-2.3, 0.2)	0.09	1.0 (-0.2, 2.2)	0.09
Haemoglobin (g/L)	114 ± 9	116 ± 9	116 ±10	-2 (-5, 1)	0.19	-1.5 (-5.1, 2.2)	0.43	0.7 (-2.9, 4.2)	0.71
PCV	0.34 ± 0.03	0.35 ± 0.03	0.35 ± 0.04	-0.01 (-0.02, 0.00)	0.10	-0.01 (-0.02, 0.01)	0.27	0 (-0.01, 0.01)	0.74
Urea (mmol/L)	2.8 ± 0.5	3.1 ± 0.6	2.4 ± 0.7	-0.3 (-0.5, -0.1)	0.01	0.4 (0.1, 0.6)	0.001	0.6 (0.4, 0.8)	<.0001
Folate (nmol/L)	30.7 ± 5.6	42.1 ± 3.9	36.5 ± 5.5	-11.4 (-13.2, -9.5)	<0.0001	-6.7 (-8.7, -4.7)	<.0001	4.7 (2.8, 6.7)	<.0001
Ferritin (µg/L)	100 ± 70	92 ± 60	114 ± 83	1.1 (0.8, 1.5)	0.65	0.9 (0.7, 1.3)	0.66	0.9 (0.6, 1.2)	0.31

GIF: goat milk infant formula; CIF: cow milk infant formula; BF: breastfed; CI: confidence interval. PCV: packed cell volume.

[1]Mean ± SD (all such values).

Table 4. Incidence of parental reports food allergy/gastrointestinal symptoms in the 12 month study period

	GIF	CIF	BF	Relative risk (95% CI) GIF vs. CIF	P	Relative risk (95% CI) GIF vs. BF	P	Relative risk (95% CI) CIF vs. BF	P
	n/N	n/N	n/N						
Wheeze/stridor	43/94	49/91	30/100	0.88 (0.66, 1.17)	0.37	1.37 (0.93, 2.03)	0.12	1.57 (1.07, 2.3)	0.02
Vomiting	81/94	79/94	79/100	1.03 (0.92, 1.15)	0.57	1.11 (0.98, 1.26)	0.11	1.09 (0.94, 1.26)	0.24
Loose watery stool	72/93	77/92	81/100	0.92 (0.8, 1.06)	0.26	0.9 (0.76, 1.07)	0.23	0.95 (0.82, 1.12)	0.56
Blood stained stools	17/90	7/86	7/100	2.39 (1.05, 5.48)	0.04	3.81 (1.67, 8.69)	0.01	1.57 (0.56, 4.42)	0.39
Itchy rash	32/91	35/87	37/100	0.87 (0.6, 1.27)	0.47	1.05 (0.7, 1.58)	0.80	1.21 (0.82, 1.78)	0.34
Other skin problems	14/91	18/87	16/99	0.76 (0.4, 1.43)	0.39	1.18 (0.56, 2.48)	0.67	1.58 (0.76, 3.27)	0.22

GIF: goat milk infant formula; CIF: cow milk infant formula; BF: breastfed; CI: confidence interval.

Figure 2. Weight (a), length (b), head circumference (c) and weight-for-length (d) z-scores of infants fed goat milk formula (triangle), cow milk formula (solid circle) or breast milk (open circle). Z-score data were based on WHO reference data and values are mean +/- SD of imputed data. * Statistically significant difference between goat formula and breast milk groups. ** Statistically significant difference between cow formula and breast milk groups. Statistically significant at $p<0.05$.

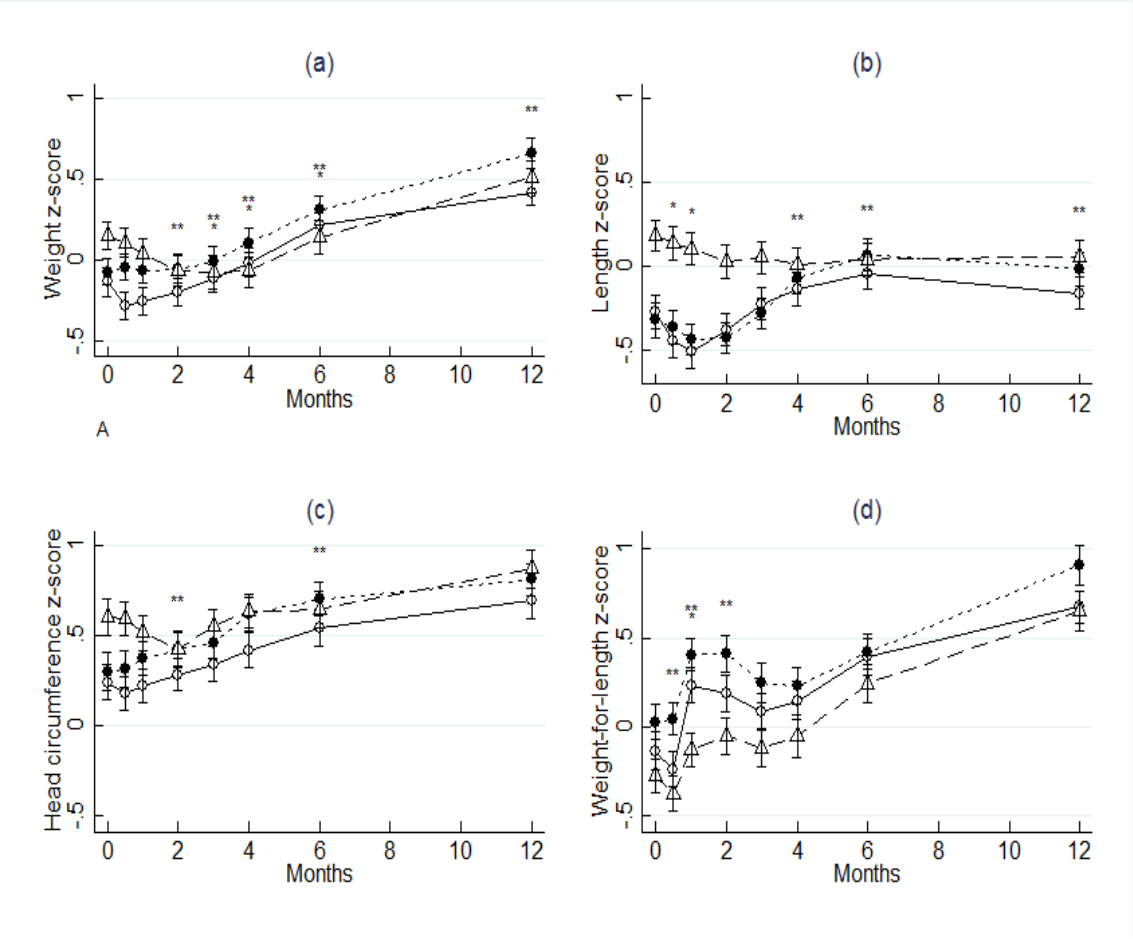
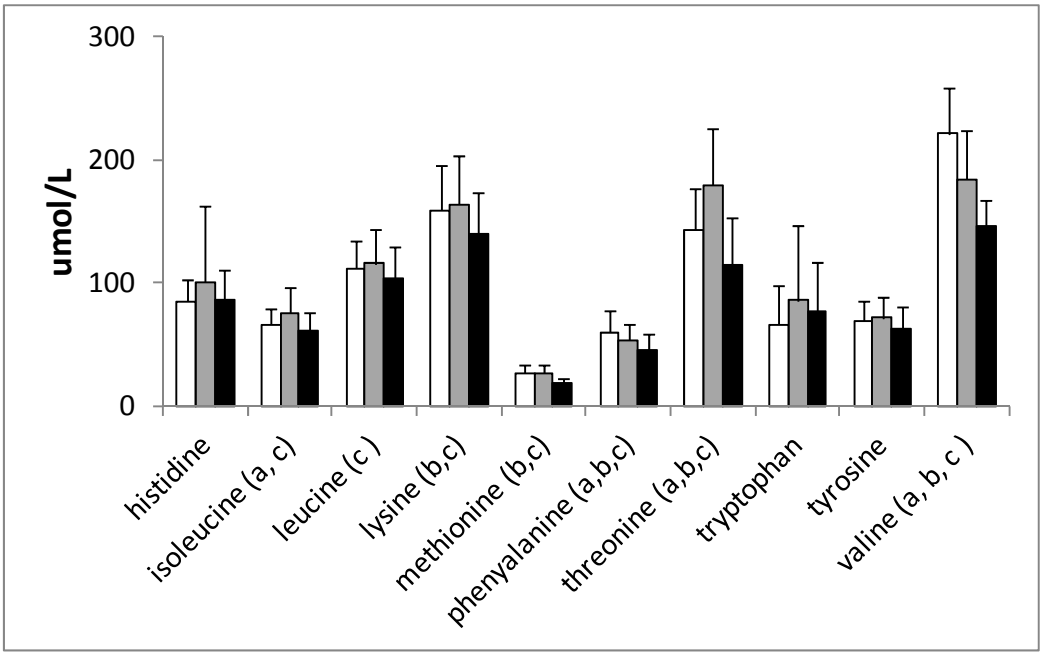


Figure 3. Mean (+/-SD) concentrations of essential and semi-essential amino acids in plasma of infants after 4 months of being fed goat milk formula (open bars), cow milk formula (gray bars) groups or breast milk (closed bars). a: significant difference between formula groups. b: significant difference goat formula and breast milk groups. c: significant difference cow formula and breast milk groups. Significant at $p<0.05$.



References

1. AAP (2012) Breastfeeding and the use of human milk. *Pediatr* **129**, e827-e841.
2. Raiha N C, Fazzolari-Nesci A, Cajozzo C*, et al.* (2002) Whey predominant, whey modified infant formula with protein/energy ratio of 1.8 g/100 kcal: adequate and safe for term infants from birth to four months. *J Pediatr Gastroenterol Nutr* **35**, 275-81.
3. Hernell O (2011) Human milk vs. cow's milk and the evolution of infant formulas. *Nestle Nutr Workshop Ser Pediatr Program* **67**, 17-28.
4. Ziegler D S, Russell S J, Rozenberg G*, et al.* (2005) Goats' milk quackery. *J Paediatr Child Health* **41**, 569-71.
5. Basnet S, Schneider M, Gazit A*, et al.* (2010) Fresh goat's milk for infants: myths and realities--a review. *Pediatrics* **125**, e973-7.
6. Taitz L S and Armitage B L (1984) Goats' milk for infants and children. *Br Med J (Clin Res Ed)* **288**, 428-9.
7. Baur L A and Allen J R (2005) Goat milk for infants: Yes or no? *J Paediatr Child Health* **41**, 543-543.
8. Rutherfurd S, Moughan P, Lowry D*, et al.* (2008) Amino acid composition determined using multiple hydrolysis times for three goat milk formulations. *Int J Food Sci Nutr* **59**, 679-90.
9. Koletzko B, Baker S, Cleghorn G*, et al.* (2005) Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. *J Pediatr Gastroenterol Nutr* **41**, 584-99.
10. Codex Alimentarius Commission (2007) Standard for infant formula and formulas for special medical purposes intended for infants CODEX STAN 72-1981 (amended 2007). .
11. Rutherfurd S M, Darragh A J, Hendriks W H*, et al.* (2006) True Ileal Amino Acid Digestibility of Goat and Cow Milk Infant Formulas. *J Dairy Sci* **89**, 2408-2413.
12. Koletzko B, Ashwell M, Beck B*, et al.* (2002) Characterisation of infant food modifications in the European Union. *Ann Nutr Metab* **46**, 231-42.
13. Silanikove N, Leitner, G., Merin, U., Prosser, C. G. (2010) Recent advances in exploiting goat's mik: Quality, safety and production aspects. *Small Rum Res* **89**, 110-124.

14. Razafindrakoto O, Ravelomanana N, Rasolofo A*, et al.* (1994) Goat's Milk as a Substitute for Cow's Milk in Undernourished Children: A Randomised Double-Blind Clinical Trial. *Pediatrics* **94**, 65-69.
15. Haenlein G F W (2004) Goat milk in human nutrition. *Small Rum Res* **51**, 155 - 163.
16. Grant C, Rotherham B, Sharpe S*, et al.* (2005) Randomized, double-blind comparison of growth in infants receiving goat milk formula versus cow milk infant formula. *J Paediatr Child Health* **41**, 564-8.
17. EFSA Panel on Dietetic Products Nutrition and Allergies (2004) Scientific Opinion on the suitability of goat milk protein as a source of protein in infant formulae and in follow-on formulae *EFSA J* **30**, 1-15.
18. Koletzko B, von Kries R, Closa R*, et al.* (2009) Lower protein in infant formula is associated with lower weight up to age 2 y: a randomised clinical trial. *Am J Clin Nutr* **89**, 1836-1845.
19. Bang H, Ni L, and Davis C E (2004) Assessment of blinding in clinical trials. *Control Clin Trials* **25**, 143-56.
20. European Task Force on Atopic Dermatitis (1993) Severity scoring of atopic dermatitis: the SCORAD index. *Dermatology* **186**, 23-31.
21. Lewis S J and Heaton K W (1997) Stool Form Scale as a Useful Guide to Intestinal Transit Time. *Scandinavian Journal of Gastroenterology* **32**, 920-924.
22. Matthey S (2001) The sleep and settle questionnaire for parents of infants: psychometric properties. *J Paediatr Child Health* **37**, 470-5.
23. World Health Organisation (2006) WHO child growth standards based on length/height, weight and age. *Acta Paediatr* **Suppl 450**, 76-85.
24. Himes R and Shulman R, *Use of laboratory measurements in nutritional assessment*, in *Pediatric Nutrition in Practice*, B. Koletzko, Editor 2008, Karger: Basel. p. 27-30.
25. Kramer M S, Guo T, Platt R W*, et al.* (2004) Feeding effects on growth during infancy. *J Pediatr* **145**, 600-5.
26. Dewey K G, Heinig M J, Nommsen L A*, et al.* (1992) Growth of breast-fed and formula-fed infants from 0 to 18 months - the Darling Study. *Pediatrics* **89**, 1035-1041.
27. Agostoni C, Grandi F, Gianni M L*, et al.* (1999) Growth patterns of breast fed and formula fed infants in the first 12 months of life: an Italian study. *Arch Dis Child* **81**, 395-9.

28. Janas L M, Picciano M F, and Hatch T F (1987) Indices of protein metabolism in term infants fed either human milk or formulas with reduced protein concentration and various whey casein ratios. *J Pediatr* **110**, 838-848.

29. Janas L M, Picciano M F, and Hatch T F (1985) Indices of protein metabolism in term infants fed human milk, whey predominant formula or cows milk formula. *Pediatrics* **75**, 775-784.

30. Lonnerdal B and Hernell O (1998) Effects of feeding ultrahigh-temperature (UHP)-treated infant formula with different protein concentrations or powdered formula, as compared with breast-feeding, on plasma amino acids, hematology, and trace element status. *Am J Clin Nutr* **68**, 350-356.

31. Sandstrom O, Lonnerdal B, Graverholt G*, et al.* (2008) Effects of {alpha}-lactalbumin-enriched formula containing different concentrations of glycomacropeptide on infant nutrition. *Am J Clin Nutr* **87**, 921-928.