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

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

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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 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 (Hitachi column) 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.
Sample size and power calculation

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

Statistical analysis

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

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

Continuous outcomes measured at multiple assessments, including the primary anthropometric outcomes, were compared between formula and breastfeeding groups over time using linear mixed effects models. Fixed effects for group, time and the interaction between group and time were included in the models, while dependence was accounted for by allowing for correlated residuals within a child. Independent of 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 analyses 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
thrust 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 + 6.7 for GIF, 11.9 + 7.1 for CIF and 11.1 + 6.3 for
breast fed groups (mean ± 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 ± 1.6, 2.0 ± 1.3, 1.6 ± 1.0 and 1.6 ± 0.9 (mean ±
SD), respectively. These were not different from the stool frequency of infants in the
CIF group, which were 2.5 ± 1.4, 2.0 ± 1.4, 1.5 ± 0.9 and 1.6 ± 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 ± 3.3, 5.0 ± 2.3, 3.0 ± 2.2
and 2.4 ± 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 ± 1.44 vs. CIF 5.46 ± 0.96, p < 0.0001) and 1 month (GIF 4.95 ± 1.35 vs. CIF
5.35 ± 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
infants fed low protein formula was not different to breast fed infants while infants fed high protein formula (2.9 g/ 100 kcal) had higher z-score. There were minor differences in the blood biomarkers between the formula fed groups, which likely reflected differences in the composition of the two formulae. For instance, the cow infant formula contained added folate close to the recommended maximum, compared with the goat milk formula that had an amount in the mid-range of the recommendations \(^{(9,10)}\). Nevertheless, concentrations of blood biomarkers measured at four months were within the normal reference range for infants of this age \(^{(24)}\).

Whey proteins are often added to formula to help improve protein quality and availability of essential and semi-essential amino acids \(^{(28,29)}\). Infant formula made from goat milk without added whey proteins was shown to have sufficient quantities of all the essential and semi-essential amino acids \(^{(8)}\) and similar digestion and absorption of the amino acids in an animal model compared with a whey based cow infant formula \(^{(11)}\). The present study shows some differences in plasma amino acids profile between the formula groups as well as in comparison with the breastfed infants, but there were large inter-individual variations. Although the differences were statistically significant, they are unlikely to be clinically important as the mean plasma amino acid concentration of infants in both formula groups are comparable with those reported in other studies \(^{(30,31)}\).

This study is the first to record a wide range of outcomes related to general health, gastrointestinal function and allergy when infants were exposed to goat infant formula using a combination of objective clinical assessments and subjective parental reports. There were no differences in objective assessments of allergy related outcomes including dermatitis and medically diagnosed food allergy. The only statistically significant finding between the formula groups was a greater number of parental reports of blood stained stools in infants fed goat compared with cow infant formula. We are unsure about the significance of this finding. Firstly, the number of reports of blood stained stools were low overall and secondly, there was no indication of other gastrointestinal disorders, differences in stool characteristics, crying and sleeping patterns, general health or other allergy-related symptoms. Furthermore, none of the infants in the study had iron deficiency anaemia which would indicate no significant blood loss over time. Finally, the outcomes related to 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.

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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
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

1180 Families screened for eligibility

200 Families randomised

101 Families randomised to Goat Milk Infant Formula

8 Families withdrew
23 families did not consent to have blood taken
93 Families completed the study
101 infants included in analysis of growth
78 infants included in analysis of blood biochemistry and plasma amino acids

99 Families randomised to Cow Milk Infant Formula

7 Families withdrew
19 families did not consent to have blood taken
92 Families completed the study
99 infants included in analysis of growth
80 infants included in analysis of blood biochemistry and plasma amino acids

101 Breastfed reference

1 Family withdrew
19 families did not consent to have blood taken
100 Families completed the study
101 infants included in analysis of growth
82 infants included in analysis of blood biochemistry and plasma amino acids
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Unit</th>
<th>Goat milk formula</th>
<th>Cow milk formula</th>
<th>Mature human milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per 100 mL</td>
<td>Per 100 mL</td>
<td>Per 100 g</td>
</tr>
<tr>
<td>Energy</td>
<td>kcal</td>
<td>65.6</td>
<td>64.8</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>kJ</td>
<td>274.0</td>
<td>271.0</td>
<td>291</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Per 100 kcal</td>
<td>Per 100 kcal</td>
<td>Per 100 g</td>
</tr>
<tr>
<td>Protein</td>
<td>g</td>
<td>2.0</td>
<td>2.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Fat</td>
<td>g</td>
<td>5.3</td>
<td>5.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>g</td>
<td>2.0</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>Unsaturated fat</td>
<td>g</td>
<td>3.3</td>
<td>3.2</td>
<td>-</td>
</tr>
<tr>
<td>Linoleic acid ω6</td>
<td>g</td>
<td>0.6</td>
<td>0.9</td>
<td>-</td>
</tr>
<tr>
<td>α-Linolenic acid ω3</td>
<td>g</td>
<td>0.1</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>g</td>
<td>11.0</td>
<td>11.0</td>
<td>6.9</td>
</tr>
</tbody>
</table>

**Vitamins**

| Vitamin A (RE)                | µg    | 141.0               | 87.0               | 61                |
| Vitamin D₃                    | µg    | 1.8                 | 2.1                | 0.1               |
| Vitamin E (TE)                | mg    | 2.6                 | 1.1                | 0.08              |
| Vitamin K₁                    | 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 B₆                    | µg    | 80.0                | 65.0               | -                 |
| Folic acid                    | µg    | 12.0                | 21.0               | 5.0³              |
| Pantothenic acid              | mg    | 0.6                 | 1.2                | 0.22              |
| Vitamin B₁₂                   | 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                | -                 |

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.

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>GIF (n=101)</th>
<th>CIF (n=99)</th>
<th>BF (n=101)</th>
<th>P-value² (FF vs. BF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>27.8 ± 6.6¹</td>
<td>28.2 ± 5.8</td>
<td>30.7 ± 5.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>Race, Caucasian [n (%)]</td>
<td>92 (91)</td>
<td>94 (95)</td>
<td>93 (92)</td>
<td></td>
</tr>
<tr>
<td>Education [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Secondary incomplete</td>
<td>30 (30)</td>
<td>36 (36)</td>
<td>10 (10)</td>
<td></td>
</tr>
<tr>
<td>Certificate/diploma or</td>
<td>65 (64)</td>
<td>58 (59)</td>
<td>50 (50)</td>
<td></td>
</tr>
<tr>
<td>secondary complete</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degree or higher degree</td>
<td>6 (6)</td>
<td>5 (5)</td>
<td>41 (41)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 ± 6.3</td>
<td>27.8 ± 7.6</td>
<td>24.6 ± 4.5</td>
<td>0.0007</td>
</tr>
<tr>
<td>Smoking in pregnancy [n (%)]</td>
<td>45 (44.6)</td>
<td>34 (34.3)</td>
<td>10 (9.9)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Infant

<table>
<thead>
<tr>
<th>Birth characteristics</th>
<th>GIF (n=101)</th>
<th>CIF (n=99)</th>
<th>BF (n=101)</th>
<th>P-value²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M [n (%)]</td>
<td>48 (47.5)</td>
<td>45 (45.5)</td>
<td>44 (43.6)</td>
<td>0.63</td>
</tr>
<tr>
<td>GA at birth (wk)</td>
<td>39.4 ± 1.0</td>
<td>39.3 ± 1.1</td>
<td>39.6 ± 1.0</td>
<td>0.048</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3379 ± 466</td>
<td>3407 ± 419</td>
<td>3564 ± 409</td>
<td>0.001</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>49.5 ± 2.0</td>
<td>49.3 ± 2.1</td>
<td>50.2 ± 2.0</td>
<td>0.003</td>
</tr>
<tr>
<td>Birth head circumference (cm)</td>
<td>34.7 ± 1.4</td>
<td>34.6 ± 1.5</td>
<td>35.1 ± 1.2</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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 | 0.001    |
| Head circumference at enrolment (cm) | 35.0 ± 1.2 | 35.1 ± 1.4 | 35.5 ± 1.3 | 0.009    |
1Mean ± SD (all such values); 2Continuous 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

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


1Mean ± SD (all such values).
Table 4. Incidence of parental reports food allergy/gastrointestinal symptoms in the 12 month study period

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

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


