

SCIENTIFIC OPINION

Scientific Opinion on the suitability of goat milk protein as a source of protein in infant formulae and in follow-on formulae¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

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ABSTRACT

On request from the European Commission following an application by Dairy Goat Co-operative (NZ) Ltd, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on the suitability of goat milk protein as a source of protein in infant and follow-on formulae. The Panel considered compositional data of an infant and a follow-on formula made from whole goat milk that retained the natural whey-to-casein ratio of goat milk, data from a double-blind, randomised, controlled, three-centre trial, and a re-analysis of the data of the trial which formed the basis of a previous evaluation of the Panel. A study in 200 Australian infants, randomised to receive an infant formula with unmodified goat milk protein or a cow milk formula exclusively for at least four months and thereafter in addition to complementary food until 12 months did not show statistically significant or clinically relevant differences in weight, length or head circumference development. The growth pattern of formula-fed infants differed, as expected, from that of the WHO growth standard in particular with respect to weight-for-length. The results of this study were supported by the results of the trial considered in the Panel's earlier assessment, in which, however, the sample size was insufficient to draw conclusions. The Panel concludes that protein from goat milk can be suitable as a protein source for infant and follow-on formulae, provided the final product complies with the compositional criteria laid down in Directive 2006/141/EC.

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KEY WORDS

Goat milk, protein, infant formula, follow-on formula, nutrition, safety, suitability.

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SUMMARY

On request from the European Commission following an application by Dairy Goat Co-operative (NZ) Ltd, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on the suitability of goat milk protein as a source of protein in infant formulae and in follow-on formulae.

In 2004, the Panel issued, on request of the European Commission, a scientific opinion related to the evaluation of goat milk protein as a protein source for infant formulae and follow-on formulae, followed by a statement in 2005, and concluded that there was insufficient evidence to establish the suitability of goat milk as a protein source in infant formulae.

For the present evaluation, compositional data of an infant and a follow-on formula made from whole goat milk that retained the natural whey-to-casein ratio of goat milk, data from a double-blind, randomised, controlled, three-centre trial, and a re-analysis of the data of the trial which formed the basis of the Panel's previous evaluation were submitted.

The Panel notes that there are compositional differences between goat and cow milk, which need to be taken into account in the manufacture of infant and follow-on formulae, particularly in the composition of caseins.

Cow milk allergy is the most frequent allergy in the first years of life and there is a high risk of cross-reactivity with goat milk protein in clinical studies, but also selective allergy to goat milk proteins only has been reported. The Panel notes that goat milk protein can induce allergic reactions. Currently, there are insufficient data available on the allergenicity of goat milk protein and it is not possible to predict the incidence and severity of adverse reaction to goat milk protein as a result of the proposed use. There are no convincing data to support that the incidence of allergic reactions is lower when feeding goat milk-based infant formula compared with cow milk-based infant formula.

A multicentre, randomised, double-blind controlled trial to compare growth rates and nutritional status of infants with exclusive feeding of infant formula based on goat milk (n=101) or cow milk (n=99) for at least four months, and continuous feeding of the formula up to 12 months in addition to complementary food, was performed between April 2008 and April 2009 in newborn infants recruited in three hospitals in Adelaide. An exclusively breast-fed reference group was included (n=101). The baseline characteristics of the participants were comparable between the two formula groups, except that in the goat milk formula group more mothers smoked during pregnancy. Breast-fed infants had a higher mean birth weight, lower maternal pre-pregnancy body mass index (BMI), lower percentage of smoking mothers and more parents with a higher level of education. Daily median intake of study formulae was not different between formula groups until four months of age.

There were no statistically significant or clinically relevant differences in weight, length or head circumference development between the two formula groups from registration to four months or from registration to 12 months. Differences in blood biochemistry between the two formula groups, which were significant for urea and folate, reflect differences in formulae composition but do not raise concern with respect to the safety and/or nutritional adequacy of the formulae. Some differences in indispensable amino acid (L-isoleucine, L-threonine, L-phenylalanine and L-valine) levels were also observed between the formula groups but none of the levels are considered to be of clinical significance.

There was no difference in the occurrence of serious adverse events in the two formula-fed groups of infants during the 12 months.

There were small differences in linear and ponderal growth parameters between infants from the two formula groups compared to a parallel group of exclusively breast-fed infants also after adjustment for the higher birth weight of breast-fed infants in this study. The differences, expressed in WHO z-scores, were small (<0.5 z-scores). The differences became smaller during the second half of the first year of life. When breast-fed infants were compared to formula-fed infants, urea levels were higher in the latter, while folate levels were lower in the goat milk formula group but within the normal range, which reflects the higher protein intake in the formula-fed infants and the lower folate content of the goat milk formula. Observed differences in plasma levels of some amino acids between formula- and breast-fed infants are most likely explained by the higher protein intake of the formula-fed infants compared to the breast-fed infants.

The Panel notes that the goat milk formula administered in this study complied with the compositional criteria of Directive 2006/141/EC. Follow-up until 12 months showed similar growth patterns in both formula groups. The growth pattern of formula-fed infants differed, as expected, from that of the WHO growth standard in particular with respect to weight-for-length. The biochemical parameters reflected what is known about the difference between formula and human milk feeding. The Panel considers that the study provides sufficient evidence to conclude that goat milk can be a suitable source of protein for use in infant and follow-on formulae.

The Panel also considered the re-analysis provided by the applicant of the data of the trial which formed the basis of the Panel's earlier evaluation. The Panel notes that the re-analysis of the data does not change the Panel's earlier conclusion that the sample size was insufficient to conclude that the goat milk formula was as safe and nutritionally adequate as cow milk formula. The Panel, however, also notes that the results of this study with respect to similarities in growth patterns and in the occurrence of adverse events between the goat and cow milk formula groups, as well as with respect to differences in growth between formula and breast-fed infants, are in conformity with the results of the multicentre trial described above.

The Panel has no data on which to base a conclusion on the safety and suitability of the follow-on formula manufactured with unmodified goat milk described in the dossier. However, given the similarity in composition of the goat milk infant and follow-on formulae, the fact that the infant formula was consumed throughout the first year of life, instead of follow-on formula, and the fact that the follow-on formula fulfils the compositional criteria laid down in Directive 2006/141/EC, the Panel considers that goat milk protein is a suitable protein source for follow-on formula in older infants who receive it in addition to complementary food.

The Panel concludes that protein from goat milk can be suitable as a protein source for infant and follow-on formulae, provided the final product complies with the compositional criteria laid down in Directive 2006/141/EC.

For goat milk protein to be used in infant and follow-on formulae, particular attention has to be given to the protein content and composition of the milk proteins, and to the amino acid content, which should in the final product be in compliance with Directive 2006/141/EC, if necessary by the addition of free amino acids in appropriate amounts.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Directive 2006/141/EC on infant formulae and follow-on formulae⁴ specifically mentions and sets criteria for formulae containing cow milk, soya protein isolates and partially hydrolysed protein as the protein sources in infant formulae and follow-on formulae.

The Dairy Goat Co-operative (NZ) Ltd (DGC) is requesting, on the basis of a new application, the inclusion of infant and follow-on formulae from goat milk, without added whey proteins, in Commission Directive 2006/141/EC. This application follows from the earlier request from the company Vitacare Ltd (Request No EFSA-Q-2003-019) and the supplementary data that were submitted subsequently to EFSA on 4 July 2005.

In line with the first opinion issued in February 2004⁵, EFSA concluded in its statement of 6 December 2005 that there was insufficient evidence to establish the suitability of goat milk as a protein source in infant formulae. The clinical study that was submitted, using a goat milk protein-based formula, was concluded to be insufficient due to methodological flaws such as insufficient sample size, absence of a breast-fed reference group and the fact that the protocol was not strictly followed⁶.

The technical dossier for this application prepared by the dairy Goat Co-operative contains:

- Data for a formula that was manufactured from whole goat milk which retains the natural whey casein ratio in goat milk;
- Data which are presented to confirm that the formula complies with the macro and micro-nutrient requirements and the amino acid profile required by Directive 2006/141/EC;
- Evidence from a clinical trial on the safety and nutritional adequacy of the formula for infants from birth to 12 months.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of the above, and in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the Commission asks EFSA to give a scientific opinion on the basis of the new application and the current scientific knowledge on the suitability of goat milk protein as a source of protein in infant formulae and in follow-on formulae.

If the goat milk protein under evaluation is considered to be suitable as a source of protein in infant formulae and follow-on formulae, EFSA is asked to advise on any condition for use that might be necessary.

⁴ OJ No L 401, 30.12.2006, p. 1

⁵ <http://www.efsa.europa.eu/en/scdocs/scdoc/30.htm>

⁶ See minutes' Statement of the Scientific Panel of Dietetic Products, Nutrition and Allergies replying to applicant's comment on the Panel's Opinion relating to the evaluation of goat's milk protein as a protein source for infant formulae and follow-on formulae (expressed on 6 December 2005 at its 12th Plenary meeting, corresponding to item 8.1 of the agenda.

ASSESSMENT

1. Introduction

In 2004, the European Food Safety Authority (EFSA), issued on request of the European Commission, a scientific opinion related to the evaluation of goat milk protein as a protein source for infant formulae and follow-on formulae (EFSA, 2004) and concluded that unmodified goat milk protein and infant formula manufactured with it showed deficiencies of L-cysteine and L-tryptophan, and did not comply with Directive 91/321/EEC⁷, as amended by Directive 96/4/EC with respect to the amino acid pattern within the range of protein content permitted. New data on the amino acid pattern of goat milk infant formula, which were provided for the years 1999 to 2003 to the NDA Panel showed that the requirements of Directive 91/321/EEC with respect to the content of indispensable and conditionally indispensable amino acids per energy value according to Annex V were fulfilled. No convincing data were submitted to support that the incidence of allergic reactions was lower when feeding goat milk-based infant formula compared to cow milk-based infant formula.

However, the NDA Panel considered the data from a pilot growth study (Grant et al., 2003; 2005) (Auckland RCT) to be insufficient to establish the nutritional adequacy and safety of goat milk protein as a protein source for infant formulae and follow-on formulae because of insufficient sample size (EFSA, 2005).

On the advice of paediatric experts in nutrition, a further randomised controlled study (Adelaide RCT) following current scientific standards and including biochemical analysis of blood was planned and carried out by the applicant using a goat milk formula which was slightly modified compared to the formula used in the Auckland RCT. Moreover, a re-evaluation of the data of the Auckland RCT was performed.

The following new information was provided by the applicant:

- Compositional data of an infant and a follow-on formula made from whole goat milk that retains the natural whey-to-casein ratio of goat milk, including information on the manufacturing process.
- Data from a double-blind randomised three-centre trial (Tolerance of Infant Goat Milk Formula and Growth Assessment, TIGGA, Adelaide RCT) (Zhou et al., 2010).
- A re-analysis of the Auckland RCT up to the age of 168 days with particular consideration of a) the extent of protocol violation, i.e. contribution of not-permitted food to total daily energy intake and b) the calculation of z-scores for weight and length for each infant using the standards developed from the WHO Multicentre Growth Reference Study.

Commission Directive 2006/141/EC⁸ on infant formulae and follow-on formulae defines the essential composition of infant formulae, including accepted protein sources, in Annex I and stipulates that for other food ingredients their “suitability for particular nutritional use by infants from birth” has to be “established by generally accepted scientific data”. Neither cow nor goat milk are suitable for feeding infants without modification of the protein and mineral content (EFSA, 2004) and both need modifications of the lipid composition by addition of vegetable oils to comply with the requirements for particular fatty acids of young infants. For infant and follow-on formulae, minimum and maximum

⁷ Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae. OJ L 175, 4.7.1991, p. 35–49.

⁸ Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC Text with EEA relevance. OJ L 401, 30.12.2006, p. 1–33

levels of protein and minimum levels of certain amino acids per energy value have been established based on human milk protein as reference. Modifications of the permitted protein sources can be made by changing the natural ratio of certain protein components, for example, the ratio between the whey proteins and caseins of milk, and/or by adding in the free form those indispensable amino acids which are below the requested amount per energy value of the formula.

The applicant proposes to use unmodified goat milk as an ingredient in the manufacture of infant and follow-on formula to which other ingredients, including amino acids, are added as required to comply with the compositional criteria of Directive 2006/141/EC.

2. Nitrogen components of goat milk

The content of nitrogen components in goat milk varies according to breed, genetics, season, stage of lactation and feed (Park, 2007; Park et al., 2007). More than 95 % of the proteins contained in ruminant milk are synthesised from six structural genes that encode well characterised proteins, the whey proteins, β -lactoglobulin and α -lactalbumin, and the caseins, α_{s1} -casein, α_{s2} -casein, β -casein and κ -casein. In goats, a high polymorphism has been found at the four casein genes with several alleles associated with null or reduced expression of the respective protein. The gene CSN1S1 which codes for α_{s1} -casein is the most variable casein gene both on qualitative and quantitative terms. On the basis of the α_{s1} -casein content in milk, CSN1S1 alleles can be classed into four groups, strong alleles with α_{s1} -casein content in milk of 3.5 g/L, intermediate alleles with 1.1 g/L, weak alleles with 0.5 g/L and null alleles (O_1 , O_2 and N) with no production of α_{s1} -casein (Caroli et al., 2007; Ceballos et al., 2009; Martin et al., 2002; Neveu et al., 2002; Park et al., 2007). High levels of α_{s1} -casein are associated with a higher total protein and casein content and a higher lipid content (Manfredi et al., 1999) whilst α_{s1} -casein deficiency is associated with a higher proportion of total lipids as phospholipids and a smaller size of milk fat globules (Neveu et al., 2002). Casein micelles are generally small in goat milk due to high calcium and phosphorus concentrations. The applicant uses goat milk with low levels of α_{s1} -casein (4 g/L) compared to cow milk (7 g/L) but the goats have not been genotyped for expression of CSN1S1. The milk has a natural whey-to-casein ratio of about 20:80. Total non-protein nitrogen content is around 5-8 % of total nitrogen (Prosser et al., 2008). The main components of the non-protein nitrogen fraction are urea (30 %), free amino acids (with taurine, L-glycine, L-glutamic acid and L-glutamine being the most abundant), nucleosides, nucleotides and polyamines (Park et al., 2007; Prosser et al., 2008).

Due to the great variability in the protein composition of goat milk, careful control of the amino acid pattern of protein used in the manufacture of formula is important. Data from amino acid analyses of goat and human milk protein performed by the same authors (Darragh and Moughan, 1998; Rutherford et al., 2008) on samples from New Zealand using the same analytical method indicate a lower L-cysteine, L-isoleucine and L-tryptophan content per gram of protein (Nx6.25) in goat milk compared to human milk.

3. Differences in protein composition between goat and cow milk

The average protein content of goat milk can be higher than in cow milk, but both the protein content and the composition of proteins vary widely and are dependent on the breed, the stage of lactation, feeding, climate, parity and season, while the influence of the breed on the protein composition of cow milk is less pronounced. There is high homology (84-95 %) between the amino acid composition of the six major proteins of cow and goat milk: for example, goat β -lactoglobulin differs at six positions from bovine β -lactoglobulin which results in physicochemical and immunological differences; goat α -lactalbumin differs from other α -lactalbumins by being devoid of L-methionine and there are 12 amino acid differences between caprine and bovine α -lactalbumin; goat κ -casein differs from bovine κ -casein by having 171 instead of 169 amino acids (Jenness, 1980) and the

caseino macropeptide resulting from chymosin splitting of the κ -casein is devoid of aromatic amino acids and of L-arginine in all ruminant milks. Whilst about 50 genetic variants have been described for bovine milk proteins, the number of genetic variants for goat milk proteins is higher, and is particularly high for α_{s1} -casein. This high genetic variability in goat milk proteins, resulting in different allergenicity, may explain the inconsistent results with respect to tolerance of goat milk by subjects allergic to cow milk protein (Ballabio et al., 2011). Thirty percent of amino acids are glycosylated in goat milk compared to 60 % in bovine milk. The contents of minor proteins, lactoferrin, transferrin and prolactin are similar in goat and cow milk, but the content of folate binding protein is higher in goat than in cow milk (12 vs. 8 $\mu\text{g/mL}$). Total non-protein nitrogen content is usually somewhat higher in goat milk (5-8 % of total nitrogen) than in cow milk. The composition of the non-protein nitrogen of goat milk differs from that of cow milk, showing higher levels of total nucleotide monophosphates, free amino acids, urea, and sialic acid (Prosser et al., 2008).

3.1. Significance of the differences in protein composition for allergenicity

The Panel notes the differences, particularly in the composition of the caseins, between goat and cow milk. The digestibility of an infant formula based on goat milk protein has been assessed in animal models in comparison to infant formula based on cow milk protein, and no difference in digestibility was observed (EFSA, 2004). Cow milk allergy is the most frequent allergy in the first years of life and there is a high risk of cross-reactivity with goat milk protein in clinical studies (Ballabio et al., 2011; Infante Pina et al., 2003), but also selective allergy to goat milk proteins only has been reported (Muñoz Martín et al., 2004). Reactivity of goat milk protein in immunoblotting with monoclonal antibodies or serum from six cow milk allergic children and in skin prick tests depended in one report on α_{s1} -casein genetic polymorphisms with a lower reaction with goat milk devoid of or low in α_{s1} -casein content. However, the α_{s1} -casein content was not the only determinant of the reactivity of the serum of some individuals analysed by immunoblotting (Ballabio et al., 2011). The Panel notes that goat milk protein can induce allergic reactions. Currently, there are insufficient data available on the allergenicity of goat milk protein and it is not possible to predict the incidence and severity of adverse reaction to goat milk protein as a result of the proposed use. There are no convincing data to support that the incidence of allergic reactions is lower when feeding goat milk-based infant formula compared with cow milk-based infant formula. The Panel notes that substituting goat milk protein for cow milk protein in infant formula intended for cow milk allergic infants cannot be considered safe unless proven to be so in clinical and *in vitro* studies.

4. Manufacturing of goat milk infant formula

The applicant has provided a flow chart of the process and process controls applied for the manufacture of infant and follow-on formulae from fresh goat milk and indicated the Hazard Analysis and Critical Control Points (HACCP) and sampling points for compositional, microbiological and functional analyses.

Briefly, fresh milk is sampled for microbiological and compositional analyses, cooled, and then pasteurised; amino acids (L-isoleucine, L-tryptophan), minerals, vitamins, vegetable oils, lecithin and lactose are added to the liquid product; after heating, concentration and homogenisation the concentrate is filtered, spray dried to yield a powder, which is sifted and passed through magnets, flushed with nitrogen and passed through a metal detector, thereafter packed in bulk bins and released for dry-blending with lactose and canning after several final product controls and analyses.

The applicant states that organoleptic properties and nutrient values are retained over 36 months of shelf-life.

5. Composition of study formulae in the Adelaide RCT

The composition of the goat milk and the cow milk infant formulae tested in the Adelaide RCT is given in Table 1. The energy value of the tested goat milk infant formula was 66 kcal/100 mL and the protein content 2.0 g/100 kcal (total N \times 6.25) with a whey-to-casein ratio of 20:80. The formula was made from the following ingredients: 43 % goat milk solids, lactose, vegetable oils (canola, high-oleic acid sunflower and sunflower oils), minerals, acidity regulator (citric acid), vitamins, choline chloride, L-isoleucine, L-tryptophan, taurine and L-carnitine.

The goat milk infant formula complied with the compositional criteria of Directive 2006/141/EC.

The ingredient list of the cow milk infant formula studied in the Adelaide RCT is: cow milk solids (demineralised whey, skimmed milk solids, whey solids, whey protein concentrate to achieve a whey-to-casein ratio of 60:40), lactose, unspecified vegetable oils, soy lecithin, L-tryptophan, L-tyrosine, taurine and acidity regulator (citric acid and/or calcium hydroxide).

Table 1 Composition of the study formulae in the Adelaide RCT

Nutrient	Unit	Infant formula Directive 2006/141/EC		Adelaide goat infant formula	Adelaide cow infant formula
		Min	Max		
Energy	kcal/100 mL	60	70	66	65
		Per 100 kcal	Per 100 kcal	Per 100 kcal	Per 100 kcal
Protein	g	1.8	3.0	2.0	2.1
Fat	g	4.4	6.0	5.3	5.2
Linoleic acid	g	0.3	1.2	0.6	0.9
α -Linolenic acid	mg	50	Not specified	83	134
Carbohydrates	g	9.0	14	11	11
Folic acid	μ g	10	50	12	21
Cyst(e)ine	mg	38		22	40
Histidine	mg	40		55	48
Isoleucine	mg	90		102	126
Leucine	mg	166		193	215
Lysine	mg	113		149	165
Methionine	mg	23		43	43
Phenylalanine	mg	83		94	82
Threonine	mg	77		98	115
Tryptophan	mg	32		39	37
Tyrosine	mg	76		73	79
Valine	mg	88		138	126
Cystine+methionine	mg			65	82
Tyrosine+phenylalanine	mg			167	161
Methionine:cystine ratio				1.98	1.08
Tyrosine:phenylalanine ratio				0.78	0.96

6. Nutritional safety and nutritional adequacy of goat milk infant formula in infants

6.1. Tolerance of Infant Goat Milk Formula and Growth Assessment, TIGGA (Adelaide RCT)

A multicentre, randomised, double-blind controlled trial to compare growth rates and nutritional status of infants with exclusive feeding of infant formula based on goat milk or cow milk for at least four months was performed between April 2008 and April 2009 in newborn infants recruited in three hospitals in Adelaide (Zhou et al., 2010). An exclusively breast-fed reference group (up to at least four months) was included.

The primary outcomes were body weight, length and head circumference at enrolment and at the indicated time points.

The secondary outcome was markers of nutritional status in blood at the age of four months (haemoglobin, haematocrit, creatinine, urea nitrogen, folate, albumin, ferritin, blood amino acids).

Midwives in the post-natal wards identified exclusively formula-fed or exclusively breast-fed infants. Their parents were approached by study personnel who screened 1,180 families for eligibility to participate of whom 768 were eligible and of whom 301 consented to take part in the study. Two hundred infants were formula-fed and 101 were breast-fed. From 101 infants randomised to goat milk formula, between 82 and 98 could be measured at different time points, from the 99 infants on cow milk formula this number was between 75 and 98, and from the 101 breast-fed infants it was between 99 and 100. Blood at four months of age could be obtained from about half of the infants (about 40 % in the breast-fed group).

Inclusion criteria were exclusive formula feeding at the age of 14 days and consent to randomisation to either of the two infant formulae, or breast-feeding at the age of 14 days and intention to breast-feed exclusively for at least four months, and healthy infants born after 37-42 weeks of gestation with a birth weight ≥ 2.50 and ≤ 4.75 kg. Exclusion criteria were severe congenital or metabolic diseases likely to affect infant feeding or growth, and multiple births.

Infants in the formula group were randomly (computer-generated schedule with balanced variable blocks stratified for centre and sex) allocated to either a goat or a cow milk formula which were provided free-of-charge under four colour codes (two different colours per formula) within 14 days from birth. Both investigators and parents were blind to the formula given until the end of the trial. The Panel notes that it cannot be excluded that the caregivers were unblinded to the formulae.

Infants were to be fed the trial formula exclusively (no other liquids or solids except water, vitamin or mineral supplements or medicines) until at least four months of age, and to continue with the infant formula thereafter in addition to complementary food until 12 months of age. The decision to start complementary food between four and six months of age was made by the caregivers.

Weight, length and head circumference were measured at enrolment, at 2 weeks, and at 1, 2, 3, 4, 6 and 12 months. General health and symptoms of eczema were assessed regularly (2 weeks, 1, 2, 3, 4, 6 and 12 months). Formula intake (amount of formula prepared minus amount discarded), formula tolerance, stool frequency and adverse health events were assessed at each visit from parents' diaries up to four months. No list of (serious) adverse health events to be reported was provided.

Blood was analysed at the age of four months for markers of nutritional status (haemoglobin, haematocrit, creatinine, urea nitrogen, folate, albumin, ferritin, amino acids).

Non-compliance was assessed as the cumulative frequency of non-compliant days during the first four months by parental reports. Days on which formula other than the allocated formula and/or milk, juices, solids or semi-solid foods (more than one teaspoon) were given were counted and the infant was considered as non-compliant with the intervention when more than 12 non-compliant days were reported between the age of 14 days and four months. Such infants were, however, included in the intention-to-treat (ITT) analysis.

The necessary sample size was calculated to be 64 per feeding group to detect a difference in weight gain equal to 0.5 SD at 80 % power with an $\alpha=0.05$. An SD of 4.6 g/day for body weight gain had been found in the pilot growth study (Grant et al., 2003; 2005). Anticipating an attrition of 10-20 %, 100 infants per feeding group were recruited.

The analyses of the results from the primary and secondary outcomes were done blinded to treatment group. Descriptive statistics were provided for the characterisation of the randomised study groups, for missing data and for serious adverse events per treatment group.

Both ITT and per protocol (PP) analyses were performed for the comparison between formula groups and for the pair-wise comparison between the breast-fed reference group and the formula groups. Data from infants which were withdrawn from the study are included up to the time of withdrawal and data collected thereafter with permission. In the case of missing data, multiple imputations were done using linear regression models for normal outcomes and logistic regression models for binary outcomes to create 50 complete datasets for analysis. Sensitivity analyses adjusting for centre and for maternal smoking in the comparison of the two formulae were performed using different imputation models and using the raw data only. Outliers were not excluded from the primary analysis. Conclusions on the effectiveness of the feeding regimen were based on adjusted comparisons of the two randomised groups using an ITT approach.

Average weights, lengths and head circumferences for each child at every time point (registration, 2 weeks, 1, 2, 3, 4, 6 and 12 months) were compared as well as the average gains in weight, length and head circumference from registration to four and six months. Individual weight, length and head circumference values were converted to z-scores using WHO child growth standards (WHO Multicentre Growth Reference Study Group, 2006) and WHO software (WHO, 2009) which already incorporate the gender of the infant and the age. Differences in mean measurements and in z-scores per formula group were compared using a linear mixed effects model, with treatment group, time and interaction between these as fixed effects and correlation within individuals as random effect. Separate estimates of treatment effects were obtained at each time point, independent of whether the interaction was statistically significant or not. On request from EFSA, the applicant performed separate analyses for the time between registration and four months, and the time between registration and 12 months, of both raw data and calculated z-scores after adjustment for the corresponding raw birth measurements and centre, and for birth z-scores and centre, respectively.

For each variable, statistical significance at the 0.05 level was assessed using a two-sided comparative test. For binary outcomes, the number and percentage of subjects with the outcome of interest was examined. If the number was too small for the intended analysis, a Fisher's exact test was performed instead. For analyses performed using a log binomial model, a log Poisson general estimating equation was used if the model failed to converge. No adjustment for multiplicity of the primary outcomes and the larger number of secondary outcomes or for the multiple comparisons in the course of the study was performed. The applicant considered that adjustment for the three primary endpoints, weight, length and head circumference, was not necessary as these outcomes were interrelated, and that the fact that the results of comparisons of multiple outcomes were similar would have rather strengthened this conclusion.

To test the effect of variables other than the feeding regimen on the primary outcome, both unadjusted and adjusted analyses were done. In the groups randomised to the formulae adjustment for study

centre and sex, and for maternal smoking during pregnancy, were done and in the analyses involving the breast-fed reference groups adjustment for maternal education.

The baseline characteristics of the participants were comparable between the two formula groups, except that in the goat milk formula group more mothers smoked during pregnancy. Breast-fed infants had a higher mean birth weight, lower maternal pre-pregnancy BMI, lower percentage of smoking mothers, and more parents with a higher level of education.

Of the goat milk group, 73 %, and of the cow milk group, 60 %, were compliant with feeding the allotted formulae exclusively until the age of four months, and 75.2 % of the breast-fed infants were breast-fed exclusively for this period.

There was no difference in the occurrence of serious adverse events leading to hospital admission and considered to be possibly related to the type of feeding in the two groups of formula-fed infants during 12 months. These were: cyanosis after vomiting, failure to thrive and apnoea (goat milk formula); eczema exacerbation, poor feeding plus plethora and oral thrush, feeding difficulties and vomiting (cow milk formula). None of the serious adverse events leading to hospital admission reported in the breast-fed infants was considered to be related to the type of feeding.

Daily median intake of study formulae ranged from 698 (interquartile range 570-825) mL in the first two weeks to 1000 mL (855-1190 mL) at four and six months of age without significant differences in formula intake between the different formula groups until four months of age. Some non-trial feeds were given at (median, interquartile range) 2 (1-10) weeks in the goat milk formula group and at 3 (1-15) weeks in the cow milk formula group.

Gains in weight, length and head circumference were not statistically significantly different between the formula groups from enrolment to four or six months neither in the ITT nor in the PP analysis with or without adjustment for centre and sex, nor did inclusion of imputed data make a difference.

There were no statistically significant differences in absolute weights and weight z-scores between the two formula groups from registration to four months (both ITT and PP analysis), except for the time point two weeks (difference of means (goat milk minus cow milk) -97.46 g (95 % CI -152.9, -42.1) and -0.29 z-scores (95 % CI -0.38, -0.02)), and from registration to 12 months, except for the time point 12 months (z-score only, -0.20 (95 % CI -0.39, -0.01)). There were no statistically significant differences in absolute lengths and length z-scores from 0 to 4 and from 0 to 12 months in the ITT and PP analyses. There were inconsistent results with respect to differences in head circumference z-scores at different time points. However, when this group difference was statistically significant at four months, the absolute head circumference values (mean±SD) were 41.57±1.3 cm in the goat milk formula group and 41.79±1.19 cm in the cow milk formula group, a difference which may be considered of no biological significance. There were no significant differences for weight-for-length z-scores between the two formula groups over the duration of exclusive formula feeding or during follow-up until 12 months of age, neither in the ITT nor in the PP analysis, with or without adjustment for centre. Nor did adjustment for maternal smoking or inclusion of imputed missing data make a difference.

The serum concentrations of albumin, creatinine, haemoglobin, haematocrit and ferritin did not differ between infants from the formula groups, whilst serum urea and folate levels were lower in the goat milk formula group. The lower folate level is explained by the lower folate concentration in the goat milk formula. This lower concentration does not raise concern as the level is within the reference range (mean±SD (PP analysis) 29.98±5.27 nmol/L (goat milk formula group) vs. 41.93±1.91 nmol/L (cow milk formula group); reference range 5-45 nmol/L).

The analysis of plasma amino acids, performed at the age of four months, did show some differences in indispensable amino acid levels between the goat milk and the cow milk formula group which were

statistically significant both in the ITT and the PP analysis of raw, adjusted and imputed data: L-isoleucine and L-threonine were lower in the goat milk formula group than in the cow milk formula group (raw mean \pm SD 0.066 \pm 0.013 vs. 0.076 \pm 0.021 nmol/mL and 0.143 \pm 0.033 vs. 0.179 \pm 0.047 nmol/mL, respectively) and L-phenylalanine and L-valine were higher in the goat milk group (raw mean \pm SD 0.060 \pm 0.017 vs. 0.054 \pm 0.012 nmol/mL and 0.221 \pm 0.038 vs. 0.184 \pm 0.039 nmol/mL). The Panel notes that blood sampling was not standardised with respect to the interval between feeding and sampling, and with respect to the amount of feeding, and that plasma amino acid levels can be influenced by the timing of blood sampling in relation to the last meal. No information on the frequency of meals was provided. None of the levels are considered to be of clinical significance. The Panel also notes the absence of information on L-cysteine levels in the submitted data.

In the breast-fed group, some supplementary feed was given at a later age (15 (7-20) weeks; median, interquartile range) compared to the formula groups.

In the PP analysis using mixed effects models (raw adjusted data) and involving 74 infants in the goat milk formula group, 59 infants in the cow milk formula group and 76 breast-fed infants, the weight-for-age z-scores of the formula-fed infants were lower until the age of three months compared to those of the breast-fed infants and were significantly higher ($p\leq 0.001$) from four months of age until 12 months of age. The difference was less than 0.5 z-score at all time-points (see Figure 1). The same significant effect was seen in the ITT analysis with higher p-values. Gain in weight was significantly higher in the PP analysis adjusted for maternal education, birth weight and birth weight z-scores for infants in the goat milk formula and cow milk formula groups compared with breast-fed infants from registration to four or six months of age.

PP analysis of z-scores for length-for-age data showed significantly lower values at age two weeks and one month in the goat milk formula group compared with breast-fed infants. No significant differences at later time points were observed, whilst the values in the cow milk formula group were higher from four months of age. Gain in length was higher in the goat milk formula group from registration to four or six months of age compared with the breast-fed infants in both the adjusted PP and ITT analysis.

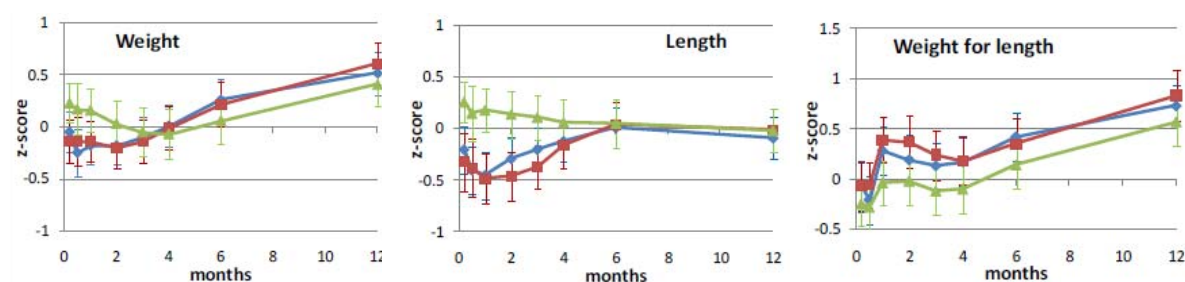


Figure 1 Mean z-scores (with 95 % CIs) (raw means from PP analysis) for weight, length and weight-for-length in the goat milk infant formula group (n=74, \blacklozenge) and cow milk infant formula group (n=59, \blacksquare) and in the breast-fed infants (n=76, \blacktriangle) at baseline (2 weeks of age) and at 1, 2, 3, 4, 6 and 12 months of age.

The weight-for-length z-scores of the goat milk and cow milk formula groups were significantly higher compared with the breast-fed group from age one to six months in the adjusted PP analysis, whilst in the adjusted ITT analysis the difference was only significant at one month and both one and two months in the goat milk and cow milk formula group, respectively.

Head circumference did not differ between infants in the goat milk formula group and breast-fed infants, while infants in the cow milk formula group had significantly higher z-scores at four and six months of age (both PP and ITT analysis).

When breast-fed infants were compared to formula-fed infants, urea levels were higher in the latter, while folate levels were lower in the goat milk formula group but within the normal range, which reflects the higher protein intake in the formula-fed infants and the lower folate content of the goat milk formula.

Plasma amino acids were different between formula- and breast-fed infants. Infants in the goat milk formula group showed statistically significant higher plasma levels of the amino acids L-lysine, L-methionine, L-phenylalanine, L-threonine and L-valine than breast-fed infants in both the ITT and PP analysis of raw, adjusted and imputed data. Infants from the cow milk formula group showed statistically significant higher plasma levels of L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine and L-valine than breast-fed infants in both the ITT and PP analysis of raw, adjusted and imputed data. The Panel notes that blood sampling was done only once and was not standardised with respect to the interval to the last meal and to the amount of feeding, and that the observed differences in the plasma levels of some amino acids between formula- and breast-fed infants are most likely explained by the higher protein intake of the formula-fed infants compared to the breast-fed infants. Differences in the plasma amino acid levels between formula-fed and breast-fed infants of the magnitude observed in this study do not permit a conclusion on the quality of the study formulae.

In summary, a study in 200 Australian infants who were randomised to receive an infant formula with unmodified goat milk protein or a cow milk formula exclusively for at least four months, and thereafter in addition to complementary food until 12 months, did not show statistically significant or clinically relevant differences in weight, length or head circumference development. The Panel notes some flaws in the conduction of the study (possibility of unblinding of caregivers) and in the statistical treatment of data (comparative analysis instead of equivalence testing, no adjustment for multiple endpoints nor for multiple comparisons).

Differences in blood biochemistry between the two formula groups, which were significant for urea and folate, reflect differences in formulae composition but do not raise concern with respect to the safety and/or nutritional adequacy of the formulae. Some differences in indispensable amino acid (L-isoleucine, L-threonine, L-phenylalanine and L-valine) levels were also observed between the formula groups but none of the levels are considered to be of clinical significance.

There was no difference in the occurrence of serious adverse events in the two groups of formula-fed infants during 12 months.

There were small differences in linear and ponderal growth parameters between infants from the two formula groups compared to a parallel group of exclusively breast-fed infants also after adjustment for the higher birth weight of breast-fed infants in this study. The differences, expressed in WHO z-scores, were small (<0.5 z-scores). The differences became smaller during the second half of the first year of life.

The Panel notes that the goat milk formula administered in this study complied with the compositional criteria of Directive 2006/141/EC. Follow-up until 12 months showed similar growth patterns in both formula groups. The growth pattern of formula-fed infants differed, as expected, from that of the WHO growth standard in particular with respect to weight-for-length. The biochemical parameters reflected what is known about the difference between formula and human milk feeding.

The Panel considers that the study provides sufficient evidence to conclude that goat milk can be a suitable source of protein for use in infant and follow-on formulae.

6.2. Goat Milk Infant Formula Growth Rate Pilot Study (Auckland RCT)

This study was performed to investigate whether feeding infant formula manufactured from goat milk was nutritionally equivalent to feeding infant formula manufactured from cow milk (Grant et al., 2003; 2005).

Of the 72 infants randomised, 62 completed the study. The birth weight and gender distribution in both groups were comparable. Daily intake of formula volume throughout the study was higher in the cow milk formula group but the difference was significant only during the last four weeks of the trial (1000 mL vs. 820 mL/day; $p=0.03$). When volume was corrected for the higher energy value of the goat milk formula, the daily energy intake throughout the period of exclusive formula feeding differed by only 0.3 %.

No statistically significant difference was seen in mean weight, length or head circumference increase between the two formula groups (EFSA, 2004, 2005).

For the present application, this study was re-analysed and the following additional data were presented:

1. The growth parameters of all infants ($n=36-33$ and $n=36-34$ in the goat and cow milk formula groups, respectively) were used in the analysis of growth parameters (weight and length) with the WHO growth standard as a reference. Growth data were also compared to a cohort of exclusively breast-fed infants, graphically and statistically, using the WHO growth standard as a reference. z-Scores for weight and length of the study population during 168 days were within one standard deviation of the age-specific reference value for fully breast-fed infants for both formula groups, and the difference in z-scores between formula groups was less than 0.3 at all time points.
2. More details on serious adverse events were given. None were considered to be related to the type of formula fed.
3. A calculation was performed of how much energy the complementary food started early in some infants contributed to the total individual energy intake. From the age of 14 to 112 days, average energy intake from supplementary food was below 5 % of the total energy intake. Only four infants were found to have consumed more than 10 % (11-18 %) of their daily energy intake from solid foods at visit eight (age 85-112 days), two in each formula group, which corresponded to a violation of the study protocol.

The Panel notes that the re-analysis of the data does not change the Panel's earlier conclusions that the sample size was insufficient to conclude that the goat milk formula was as safe and nutritionally adequate as cow milk formula. The Panel, however, also notes that the results of this study with respect to similarities in growth patterns and in the occurrence of adverse events between the goat and cow milk formula groups, as well as with respect to differences in growth between formula and breast-fed infants, are in conformity with the result of the Adelaide RCT described above.

7. Suitability of goat milk protein for follow-on formula

The Panel has no data on which to base a conclusion on the safety and suitability of the follow-on formula manufactured with unmodified goat milk described in the dossier. However, given the similarity in composition of the infant and follow-on formulae, the fact that the infant formula was consumed throughout the first year of life, instead of follow-on formula, and the fact that the follow-on formula fulfils the compositional criteria laid down in Directive 2006/141/EC, the Panel considers that goat milk protein is a suitable protein source for follow-on formula in older infants who receive it in addition to complementary food.

8. Conditions of use (infant and follow-on formulae)

For goat milk protein to be used in infant and follow-on formulae, particular attention has to be given to the protein content and composition of the milk proteins, and to the amino acid content, which should in the final product be in compliance with Directive 2006/141/EC, if necessary by the addition of free amino acids in appropriate amounts.

CONCLUSIONS

The Panel concludes that protein from goat milk can be suitable as a protein source for infant and follow-on formulae provided the final product complies with the compositional criteria laid down in Directive 2006/141/EC.

For goat milk protein to be used in infant and follow-on formulae, particular attention has to be given to the protein content and composition of the milk proteins, and to the amino acid content, which should in the final product be in compliance with Directive 2006/141/EC, if necessary by the addition of free amino acids in appropriate amounts.

DOCUMENTATION PROVIDED TO EFSA

Dossier submitted by the Dairy Goat Co-operative (NZ) Ltd (DGC), New Zealand to the European Commission for inclusion of infant and follow-on formulae manufactured from goat milk, without added whey proteins, in Commission Directive 2006/141/EC, February 2011.

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GLOSSARY AND ABBREVIATIONS

BMI	Body mass index
ITT	Intention-to-treat
PP	Per protocol
RCT	Randomised controlled trial
WHO	World Health Organization