FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

PROPOSAL P1007

PRIMARY PRODUCTION AND
PROCESSING REQUIREMENTS FOR RAW MILK PRODUCTS

ASSESSMENT OF POTENTIAL HEALTH BENEFITS ASSOCIATED WITH RAW MILK

Table of Contents

Summary

A large number of submitters to the Discussion Paper expressed the view that there are health benefits associated with the consumption of raw (unpasteurised) milk. FSANZ has therefore investigated the literature cited by submitters in support of their comments, to determine whether this evidence is of sufficient quality to validate the stated health outcomes.

Submitters proposed the following claims of health benefits and nutritional outcomes:

- There is an association between raw milk consumption and reduced allergy development during childhood;
- There is an association between raw milk consumption and a reduced risk of cardiovascular disease;
- The consumption of raw milk improves growth and development in children;
- Raw milk has a higher vitamin C content than pasteurised milk, and thus protects against scurvy;
- Pasteurisation destroys or decreases the content of vitamin A, B vitamins, vitamin D, and iodine in milk; and
- Pasteurisation reduces the availability of folate and/or calcium from milk.

FSANZ has reviewed the evidence cited by submitters and found that the majority of it was insufficient to support the above health benefits and nutritional outcomes. The information provided in submissions does indicate that pasteurisation decreases the vitamin C and thiamin contents of milk, however milk is not a major contributor of these nutrients to Australian and New Zealand diets, and so these decreases do not pose a nutritional risk.

A substantial body of well designed studies was presented by submitters in respect to the relationship between raw milk consumption and reduced allergy sensitisation during childhood. Because these studies were well designed, FSANZ conducted a more thorough review of the science regarding the relationship between raw milk consumption and allergy sensitisation.

In general, the findings of the studies on allergy sensitisation are consistent with a broader prevailing theory that there is a protective effect from a farming lifestyle. However it was noted that there are substantial limitations with the supporting evidence on raw milk consumption as a reason for this protective effect, with the evidence also demonstrating that other factors could explain the observed protection against allergy development. As a result, FSANZ is of the view that a specific association between raw milk consumption and the protection against allergy sensitisation has not been fully established in the currently available domain of scientific literature.

1. Introduction

On 6 August 2008 FSANZ released a Discussion Paper on Proposal P1007 – Primary Production and Processing Requirements for Raw Milk Products. In response to this Discussion Paper, a large number of submitters expressed the view that there are health benefits associated with the consumption of raw (unpasteurised) milk. Several of these submitters also cited publications in support of their comments.

The comments from submitters related to the consumption of liquid raw milk, and not to other raw milk products, such as cheese. Primarily, the benefits mentioned in submissions consisted of improvements in disease outcomes; or that raw milk was more nutritious and beneficial for health than pasteurised milk, based on the premise that pasteurisation reduces the nutrient content of milk.

FSANZ has therefore investigated the literature cited by submitters, to determine whether this evidence is sufficient to validate the stated favourable health and nutritional outcomes.

2. Scope of the Nutrition Assessment

Material has been reviewed on the following nutrition and health outcomes identified by submitters:

1. The association between raw milk consumption early in life and reduced sensitisation to allergies in later years;
2. The association between raw milk consumption and a reduced risk of cardiovascular disease;
3. The influence of raw milk consumption on growth and development;
4. Raw milk has a higher vitamin C content than pasteurised milk, and thus protects against scurvy;
5. Pasteurisation destroys or decreases the content of vitamin A, B vitamins vitamin D, and iodine in milk; and
6. Pasteurisation reduces the availability of folate and/or calcium from milk.

FSANZ has primarily restricted its assessment to the material cited in submissions, and has reviewed this information to ascertain if there was a sufficient basis for the claims stated above. It was found that the evidence was insufficient to substantiate the majority of claimed outcomes.

Only the literature cited on allergy sensitisation (point 1) was considered to be sufficiently robust to warrant further investigation. An additional search of the literature was therefore carried out by FSANZ on this issue, so that a full assessment of the evidence base could be undertaken (see Section 4).

FSANZ has determined that further investigations of the scientific literature are not warranted for the remaining claimed outcomes (see Section 5 for full details), due to the insufficient substantiation provided by the submitted evidence.

3. Literature review methodology

Thirty-three separate articles were cited in submissions to the discussion paper as support for the six nutrition and health outcomes described above. Of these articles, three publications could not be located because the references were incomplete, and so have not been included in the deliberations of this assessment report. The remaining articles were then subjected to a series of inclusion/exclusion criteria.

3.1 Inclusion/exclusion criteria

The following criteria were set as guidelines for determining what articles would be evaluated as part of this assessment:

- they had to include unpasteurised/raw milk (bovine or other ruminants only) as a variable of assessment;
- did not involve the pasteurisation of human milk;
- be published as a full report to allow critical evaluation;
- be published in peer-reviewed journals; and
- be written in the English language.

Additionally, articles were not considered if they investigated outcomes relating to the presence of pathogens or micro-organisms in milk. FSANZ has undertaken several other assessments for Proposal P1007 that specifically address the microbial risks of raw milk and raw milk products.

Individual studies are the main evidence for this assessment, although literature reviews that were cited by submitters have also been given consideration.

Of the 33 articles cited in submissions, 19 of these have been included for further assessment.

3.2 Evidence acquired in addition to submission references on raw milk and allergy sensitisation

FSANZ determined that further investigation was warranted for the evidence on allergy sensitisation. In undertaking this further assessment, FSANZ conducted a search of the available literature and screened the identified studies in accordance with the same inclusion/exclusion criteria used for articles obtained from submissions. In addition to the literature search, the reference lists of acquired articles were reviewed for any further studies that may have been missed.

The literature search was conducted using PubMed search engine, using the following keyword searches:

- Allergy AND pasteuri* AND milk
- Allergy AND farm AND milk
- Atopy AND pasteuri* AND milk
- Atopy AND farm AND milk

Limits were placed on the search: humans, English, and publications after 1990.

Using these search strategies, FSANZ obtained nine additional articles on allergy sensitisation that were not mentioned in submissions. Five of these studies were excluded on the basis that they did not directly assess raw/unpasteurised milk as a study variable. As a result, a total four additional studies from the literature search have been included in the assessment on the association between raw milk consumption and allergy sensitisation.

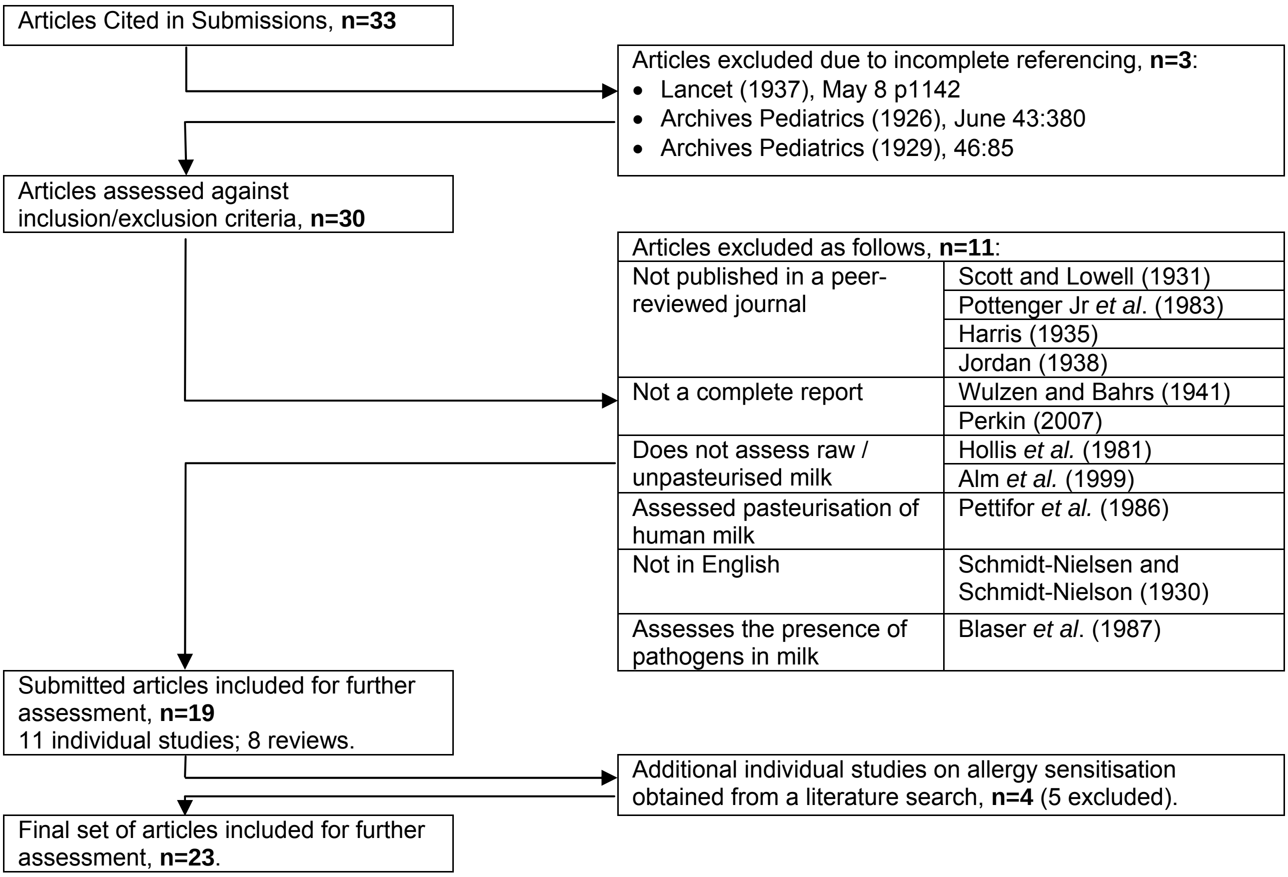

Figure 1: Exclusion and inclusion process for submitted articles, and articles from FSANZ's literature search on allergy sensitisation

3.3 Terminology and definition issues

In reviewing the evidence on the nutrition and health benefits associated with raw milk, FSANZ noted that there is inherent variation in some definitions and terms across the literature.

Within the available evidence there is the lack of a standard or agreed definition for 'raw milk'. In some studies 'raw milk' is well defined and usually refers to unpasteurised liquid bovine milk obtained from one or a few dairies (or even an allocated cattle herd). In other studies the term 'farm milk' is used, and it is not clear if this refers to milk that was obtained on a farm and kept unmodified, or if the milk was obtained on a farm and boiled before consumption. In some studies it is also unclear as to whether the milk from other species was included along with bovine milk.

The definition of pasteurisation also varies across the evidence base. While more recent studies use the standard practice of flash pasteurisation (76^{o}C for 15s), very early studies cited by some submitters (e.g. pre 1930s) occurred at a time when there was no accepted practice for pasteurisation, particularly in respect to the length of time that the milk is held at a high temperature.

FSANZ has accommodated the changes in terminology and definitions where appropriate. However, it should be recognised that comparisons between different studies are not always possible with such variations in terms and definitions.

4. Assessment of literature relating to allergy sensitisation

A body of literature has emerged since 2001 investigating, amongst other lifestyle factors, the consumption of raw milk in respect to allergy sensitisation. As mentioned above, some of the publications cited by submitters on this subject are of a high quality, and so FSANZ has investigated this field of research more thoroughly. Of the 23 studies mentioned in Figure 1 above, seven investigate allergy sensitisation (three from submissions, four from FSANZ's additional literature search). A detailed description of the characteristics and quality of the seven studies is provided in Table A1 in the Appendix.

The evidence relating to an association between raw milk and allergy sensitisation is part of a broader field of research that has been investigating the 'hygiene hypothesis'. This hypothesis proposes that the increase in allergy prevalence observed over the last few decades is due to a reduced exposure to microbes that may be more common on farms and in developing countries (Strachan, 1989). A number of studies conducted during the 1990s examined this hypothesis, and associations were found between living on a farm and a reported low prevalence of various allergic diseases (Wickens *et al*., 2002).

The seven studies identified by FSANZ were conducted with the aim of determining whether raw milk consumption (and other farming lifestyle factors) may be contributing to the associations between living on a farm and a reduced prevalence of allergic diseases. A number of the studies assessed a wide range of farming lifestyle exposures, however only raw milk consumption has been given full consideration in this assessment.

4.1 Description of studies on raw milk and allergy sensitisation

All seven studies are of a case-control design, and all but one study (Radon *et al*., 2004) investigates the consumption of raw milk during childhood years. Tables 1-3 below present the results of these studies, specifically the odds ratio (OR) results. To ensure concise tables, only the results relevant to considerations on raw milk have been included, with results for living on a farm not reported. It should be recognised that these results only report <u>associations</u> between raw milk intake and allergy outcomes; the data are insufficient to identify cause-effect relationships.

A full discussion and analysis of the OR results is provided in Section 4.2.

Table 1: Studies on raw milk and allergies - skin prick measurement of atopy
(results in bold text are statistically significant, p<0.05)

Study	Subjects		Skin Prick Results		Notes
	Groupings	n	Adjusted OR	95% CI	
A: results for milk exposures					
Barnes *et al*. (2001)	Any farm milk consumption <5 years age	n/a	0.70	0.45-1.10	
	1. No farm milk or living on farm < 5 years age	n/a	1.00	Referent*	
	2. Living on farm (no farm milk) < 5 years age	n/a	**0.56**	0.35-0.88	
	3. Farm milk + living on farm < 5 years age	n/a	**0.58**	0.34-0.98	
	4. Farm milk (no farm living) < 5 years age	n/a	**0.32**	0.13-0.78	
Perkin and Strachan (2006)	1. No early raw milk intake	3730	1.00	Referent*	
	2. Infrequent early raw milk intake	233	**0.10**	0.03-0.28	
	3. Frequent early raw milk intake	379	**0.34**	0.17-0.69	
Remes *et al*. (2003)	1. No farm milk < 1 year age	325	1.00	Referent*	The statistical significance for the trend across farm milk consumption results was p=0.73 (non-significant).
	2. Farm milk <1 times / week, < 1 year age	36	0.92	0.40-2.11	
	3. Farm milk 1-6 times / week, < 1 year age	36	0.95	0.41-2.22	
	4. Daily farm milk < 1 year age	66	1.16	0.59-2.29	
Wickens *et al*. (2002)	Pasteurised milk 1+/day < 2 years age	192	0.8	0.4-1.7	
	Unpasteurised milk ever < 2 years age	38	0.6	0.2-1.9	
	Yoghurt 1+/week < 2 years age	225	0.8	0.4-1.7	
	Cheese 1+/week < 2 years age	200	0.7	0.3-1.4	
B: results for other exposures					
Barnes *et al*. (2001)	Livestock contact <5 years age	n/a	0.72	0.49-1.07	
Perkin and Strachan (2006)	Early livestock exposure	1127	**0.71**	0.37-1.35	
	Barn/stable exposure	2340	1.02	0.93-1.13	
Remes *et al*. (2003)	1. No livestock contact < 1 year age	176	1.00	Referent*	The statistical significance for the trend across livestock contact was p=0.048 (borderline significant).
	2. Occasional livestock contact < 1 year age	358	**0.65**	0.43-0.96	
	3. Weekly livestock contact < 1 year age	113	0.73	0.41-1.28	
	4. Daily livestock < 1 year age	62	**0.46**	0.22-0.97	
Wickens *et al*. (2002)	Poultry exposure < 1 year age	36	1.1	0.4-3.5	
	Pig exposure < 1 year age	29	**0.2**	0.1-0.9	
	Cats inside house < 1 year age	223	0.6	0.3-1.3	
	Dogs inside house < 1 year age	185	0.8	0.4-1.6	

* = Referent is the exposure with an odds ratio set at 1. Other OR outcomes in the series are relative comparisons to this exposure.

Table 2: Studies on raw milk and allergies - serum IgE measurement of atopy
(results in bold text are statistically significant, p<0.05)

Study	Subjects: Groupings		Subjects: n	IgE Results (<0.35 kU/L): Adjusted OR	IgE Results (<0.35 kU/L): 95% CI	Notes
A: results for milk exposures						
Radon *et al*. (2004)	1. No Raw Milk < 6 years of age		144	1.0	Referent*	Further combinations were undertaken. Combinations with early animal contact did not decrease associations with atopy. Association with atopy decreased when *H.pylori* infection was combined with raw milk consumption.
	2. Raw milk < 6 years of age		169	0.65	0.36-1.18	
Riedler *et al*. (2001)	1. No farm milk or stable exposure in infancy		170	1.00	Referent*	
	2. Stable exposure (no farm milk) in infancy		48	0.56	0.25-1.27	
	3. Farm milk exposure (no stables) in infancy		189	**0.43**	0.24-0.77	
	4. Farm milk + stable exposure in infancy		138	**0.32**	0.17-0.62	
	5. Farm milk or stable exposure after 12 months of age		218	0.99	0.58-1.69	
Waser *et al*. (2007)	1. Shop purchased milk‡ (referent for both pollen and food allergies)		9805	1.00	Referent*	
	2. Farm milk (infancy)	- pollen allergens	1467	**0.61**	0.3-1.00	
		- food allergens	1467	0.98	0.69-1.44	
	3. Farm milk (at time of questionnaire)	- pollen allergens	869	0.73	0.42-1.27	
		- food allergens	869	0.86	0.56-1.33	
	4. Farm milk (infancy + at time of questionnaire)	- pollen allergens	2204	**0.51**	0.31-0.86	
		- food allergens	2204	**0.61**	0.42-0.89	
	5. Farm milk (infancy + at time of questionnaire) adjusted for exposure to livestock enclosures	- pollen allergens	2204	0.62	0.37-1.04	
		- food allergens	2204	**0.62**	0.42-0.91	
B: results for other exposures						
Radon *et al*. (2004)	1. Visit farm enclosure >6 years age		207	1.0	Referent*	Further combinations were undertaken. Combinations with early animal contact did not decrease associations with atopy. Association with atopy decreased when *H.pylori* infection was combined with raw milk consumption.
	2. Visit farm enclosure ≤6 years age		106	**0.49**	0.26-0.96	
	T.gondii infection		97	1.04	0.56-1.91	
	H.pylori infection		95	0.70	0.39-1.28	

* = Referent is the exposure with an odds ratio set at 1. Other OR outcomes in the series are relative comparisons to this exposure.
‡ = The descriptions of milk consumption are as reported in Waser et al (2007). It is assumed that shop purchased milk is pasteurised in this study.

Table 3: Studies on raw milk and allergies - results on self-reported allergic symptoms
(results in bold text are statistically significant, p<0.05)

Study	Subjects		Allergic Symptom Results (Adjusted OR)									
	Groupings	n	Hay-fever	95% CI	Allergic Rhinitis	95% CI	Asthma	95% CI	Current Wheeze	95% CI	Eczema / AEDS‡	95% CI
A: results for milk exposures												
Perkin and Strachan (2006)	1. No early raw milk intake	3730			1.00	Referent*	1.00	Referent*			1.00	Referent*
	2. Infrequent early raw milk intake	233			1.06	0.66-1.72	1.02	0.69-1.50			**0.54**	0.33-0.88
	3. Frequent early raw milk intake	379			0.61	0.36-1.02	0.90	0.64-1.27			**0.59**	0.40-0.87
Riedler *et al*. (2001)	1. No farm milk or stable exposure in infancy	170	1.00	Referent*			1.00	Referent*	1.00	Referent*		
	2. Stable exposure only in infancy	48	**0.25**	0.05-1.13			**0.51**	0.14-1.86	0.43	0.12-1.52		
	3. Farm milk exposure only in infancy	189	**0.24**	0.10-0.56			0.48	0.21-1.10	**0.43**	0.20-0.92		
	4. Farm milk + stable exposure in infancy	138	**0.20**	0.08-0.50			**0.14**	0.04-0.48	**0.17**	0.07-0.45		
	5. Farm milk or stable exposure after 12 months of age	218	0.88	0.44-1.74			0.88	0.42-1.86	0.60	0.28-1.28		
Waser *et al*. (2007)	1. Shop purchased milk	9805			1.00	Referent*	1.00	Referent*	1.00	Referent*		
	2. Farm milk (infancy)	2968			**0.52**	0.35-0.75	0.79	0.61-1.01	0.90	0.74-1.10		
	3. Farm milk (at time of questionnaire)	1874			**0.58**	0.36-0.94	0.74	0.54-1.02	0.85	0.61-1.17		
	4. Farm milk (infancy + at time of questionnaire)	1996			**0.61**	0.40-0.94	**0.67**	0.51-0.88	0.77	0.58-1.03		
	5. Farm milk (infancy + at time of questionnaire) adjusted for exposure to livestock enclosures	4050			0.70	0.45-1.07	**0.74**	0.56-0.98	0.82	0.61-1.10		
Wickens *et al*. (2002)	Pasteurised milk 1+/day < 2 years age	192	1.7	0.7-4.6	1.5	0.7-3.3	1.3	0.6-2.7	1.1	0.5-2.5	1.4	0.7-3.0
	Unpasteurised milk ever < 2 years age	38	1.1	0.2-5.0	0.3	0.1-1.1	0.7	0.2-2.4	0.6	0.2-0.8	0.2	0.1-2.2
	Yoghurt 1+/week < 2 years age	225	**0.3**	0.1-0.7	**0.3**	0.2-0.7	1.1	0.6-2.4	1.0	0.4-2.3	0.6	0.3-1.2
	Cheese 1+/week < 2 years age	200	2.1	0.8-5.6	1.3	0.6-2.8	1.1	0.6-2.4	1.4	0.5-3.3	1.3	0.6-2.7
B: results for other exposures												
Wickens *et al*. (2002)	Poultry exposure < 1 year age	36	1.8	0.5-6.6	2.0	0.7-5.9	2.7	0.9-7.7	2.1	0.7-6.6	**3.7**	1.3-10.7
	Pig exposure < 1 year age	29	0.4	0.1-1.9	0.6	0.2-2.0	1.0	0.3-3.3	1.0	0.3-3.3	0.6	0.2-1.8
	Cats inside house < 1 year age	223	**0.4**	0.1-1.0	1.4	0.6-3.1	0.7	0.3-1.5	1.0	0.4-2.4	**0.4**	0.2-0.8
	Dogs inside house < 1 year age	185	0.5	0.2-1.3	0.7	0.4-1.4	**0.4**	0.2-0.8	0.6	0.3-1.2	0.8	0.4-1.6

* = Referent is the exposure with an odds ratio set at 1. Other OR outcomes in the series are relative comparisons to this exposure.
‡ = AEDS: atopic eczema/dermatitis syndrome

4.1.1 Earlier studies – Barnes et al. (2001), Radon et al. (2004), Remes et al. (2003), Riedler et al. (2001), Wickens et al. (2002)

The earlier research on raw milk and allergy sensitisation consists of a group of studies that were all published within the time period of 2001-2004. In keeping with the hygiene hypothesis, all of these studies had the overarching aim of assessing the exposure to a farming environment as the basis for their design.

These studies collected data on school-aged children (range of 6-19 years), with the exception of Radon *et al*. (2004) that assessed subjects at adulthood. The prevalence of allergies and location of residence (farm versus non-farm) were assessed at the time of data collection, while farming-related variables were collected for the time when the subjects were either in infancy (Remes *et al*., 2003; Riedler *et al*., 2001) or early childhood (Barnes *et al*., 2001; Radon *et al*., 2004; Wickens *et al*., 2002). The data on early exposure to a farming lifestyle were based on questionnaires that assessed at least five years into the past.

The results on raw milk consumption vary across these early studies. Riedler *et al*. reported a significant protective association ($p<0.05$) between previous raw milk consumption and the reduced prevalence of allergies later in life (atopy and clinical symptoms of an allergy), while Barnes *et al*. and Remes *et al*. showed protective associations with atopy although these associations were not significant ($p>0.05$). Radon *et al*. and Wickens *et al*. also showed no significant associations with previous raw milk consumption, except for a protective association with atopic eczema in the Wickens *et al*. study. In commenting on their results, Radon *et al*. indicated that the non-significant associations for raw milk consumption may be the result of the low number of subjects reporting that they consumed raw milk (n=20).

The results of Remes *et al*. are notable in that the data were also analysed by the frequency of exposure to farming related variables during infancy. No variation in the prevalence of atopy later in life was observed with an increased frequency of raw milk consumption, however increasing exposure to livestock was associated with decreasing prevalence of atopy in later childhood.

In addition to the results on raw milk consumption, Wickens *et al*. reported that previous yoghurt consumption (once or more a week) had a significant ($p<0.01$) protective association with hayfever and allergic rhinitis later in life. The authors note that, in line with the hygiene hypothesis, the probiotic exposure from yoghurt may be responsible for this protective association. The results for yoghurt therefore indicate that it is possible to use a pasteurised milk product to obtain similar protective associations to those observed with raw milk consumption.

4.1.2 Recent studies – Perkin and Strachan (2006), Waser et al. (2007)

Two studies on raw milk consumption and allergy sensitisation were published recently by Waser *et al*. and Perkin and Strachan (2006). These two studies were very large in size, consisting of 14424 and 4767 subjects for Waser *et al*., and Perkin and Strachan (2006) respectively.

Both of these studies were part of larger cross-sectional studies on allergies, and involved secondary analyses of the data from these larger studies. The design of the two studies follows a similar design to the earlier studies mentioned above, with the prevalence of allergies in older school-aged children (atopy and clinical symptoms) analysed against exposures to farming lifestyle variables in earlier childhood. Both studies used questionnaires to collect information several years into the past.

The results of Perkin and Strachan (2006) show that there was a significant association ($p=0.001$) between previous raw milk consumption and the prevalence of atopy later in life (as measured via skin prick testing). A significant association ($p<0.01$) was shown between the previous consumption of raw milk and eczema, but not allergic rhinitis or asthma. The frequency of previous raw milk consumption was further analysed, however no trend was observed between higher consumption and the prevalence of the measured allergies.

Waser *et al*. showed associations between previous raw milk consumption and a reduction in the prevalence of allergies later in life, but only in respect to some of the allergy outcomes assessed. The consumption of raw milk was associated with a reduction in the prevalence of atopic sensitisations (as measured by serum IgE levels to airborne, household and food allergens), however these associations were significant ($p<0.05$) only when the consumption of raw milk at the time of the study was included. The consumption of raw milk in infancy was associated with a reduced ($p<0.05$) prevalence of rhinoconjunctivitis but not asthma or current wheezing.

In reviewing their findings, Waser *et al*. commented that the significant associations seen only with certain symptoms of allergy may be due to a bias from food avoidances, where some subjects avoid certain foods due to pre-existing allergies. When subsequent adjustments for food avoidance were made to the raw milk results, an initially significant protective association of previous raw milk consumption with eczema became non-significant ($p>0.05$).

4.2 Analysis of studies on raw milk and allergy sensitisation

4.2.1 Associations between raw milk and allergy outcomes

The data provided in Tables 1-3 above indicate that across the seven identified studies the consumption of raw milk early in life is associated with a reduced prevalence of allergies in later years. In general, early exposures to raw milk were associated with a protective effect (OR<1.0) for either atopy or self-reported allergic symptoms, and the OR were often reduced when compared with a referent (OR = 1.0) of either raw milk exposure later in life or pasteurised milk consumption.

However, there is noticeable variation in the results. For each particular measure of allergy sensitisation, the consumption of raw milk early in life did not always produce a consistent protective effect across different studies. Also, the results varied for different measures of allergy sensitisation within each individual study. Further adding to the uncertainty with the results is the wide variation in the strength of reported associations between raw milk and allergy sensitisation. Many studies often reported protective associations that had OR close to 1.0 (no effect) and wide confidence intervals.

The variations in results mentioned above were found across the evidence base, even for larger studies such as Perkin and Strachan (2006) and Waser *et al*. (2007).

4.2.2 Associations between other exposures and allergy outcomes

The associations observed between raw milk and allergies were not the only protective associations reported in the seven studies. Contacts with livestock or livestock enclosures early in life were also associated with a reduction in the prevalence of atopy and clinical symptoms of an allergy. Wickens *et al*. also reported on associations between yoghurt (pasteurised milk) and a lower prevalence of hayfever and allergic rhinitis. The strength and consistency of these associations were similar to those reported for raw milk consumption. Factors other than raw milk consumption could thus explain the lower prevalence of allergies seen with living in a farming environment, or alternatively it is the combination of several protective farming-related exposures that is important (Waser *et al*., 2007).

4.2.3 Generalisation of findings to Australia and New Zealand

All of the seven identified studies were conducted within the European region except for Wickens *et al*. (2002). Wickens *et al*. was conducted in New Zealand, and although protective associations from previous raw milk consumption were found with atopy and various self-reported allergy symptoms, these associations were statistically non-significant ($p>0.05$). The results from European studies may not therefore be wholly applicable to the New Zealand context, and may be even less applicable to Australian farming environments and conditions.

4.2.4 Limitations within the evidence base

The potential for recall bias (errors due to subject recollection) is a limitation that applies to all seven studies, and is a problem inherent with the use of retrospective designs by these studies. Recall bias could have substantially influenced the results obtained, particularly for the data on self-reported allergy symptoms that were collected years after the exposure period. Such bias could be expressed as the over-reporting of clinical symptoms by parents of children with allergies, who may have been 'searching' for explanations of their child's condition.

The inclusion of studies with a prospective cohort design in the evidence base would assist in addressing the limitation of recall bias. However, FSANZ has been unable to identify any studies with this design that examined raw milk consumption as an exposure variable.

The process of measuring raw milk intake potentially contains an inherent level of error. Australian and New Zealand infant feeding guidelines recommend against the consumption of non-human milk during infancy (Ministry of Health, 2003; NHMRC, 2003), which is consistent with European recommendations (European Commission, 2004). As a result, subjects may not have been willing to accurately report milk consumption in those (European-based) studies investigating exposures during infancy (Remes *et al*., 2003; Riedler *et al*., 2001; Waser *et al*., 2007). Also some studies had low numbers of subjects reporting that they consumed raw milk (Radon

et al., 2004; Remes *et al*., 2003; Wickens *et al*., 2002), which may have affected the statistical power for the raw milk associations.
As with all of the evidence relating to raw milk, the definition of 'raw milk' is not always made clear. In the case of the seven identified allergy studies, it is not known whether the subjects included boiled milk when reporting 'farm milk' or 'raw milk' consumption, a potential limitation recognised by Waser *et al*. (2007) in the discussion of their results. The associations seen with raw milk consumption may not therefore necessarily relate to the pasteurised status of the milk.

4.3 Conclusion for studies on raw milk and allergy sensitisation

There is some evidence for a weak association between the consumption of raw milk during early childhood and a lower prevalence of allergies later in life. However there are substantial limitations within the evidence base, most notably that the protective associations observed are variable and inconsistently reported across different measures of allergy sensitisation. The available evidence also indicates that raw milk consumption is not the only explanation for the reductions in allergy prevalence that have been observed with a farming lifestyle.

FSANZ is of the view that a specific association between raw milk consumption and the protection against allergy sensitisation has not been fully established in the currently available domain of scientific literature.

5. Consideration of remaining literature cited in submissions

The following sections discuss literature that FSANZ has determined to be insufficiently robust for demonstrating the associated claims of health benefits raised in submissions. Because this information is unable to support the claims mentioned by submitters, a broader review of the literature has not been undertaken.

5.1 Growth and development

Three studies, two on rats and one on humans, were cited in submissions to the Discussion Paper as evidence that the consumption of raw milk improves growth and development beyond that experienced with pasteurised milk. All of these studies were published in the early part of the last century (1920-1933). A summary of the studies' characteristics and their overall quality is presented in Table A2 of the Appendix to this report.

5.1.1 Daniels and Loughlin (1920)

Daniels and Loughlin conducted 19 separate experiments on a total of 122 rats. The results show that feeding rats with milk pasteurised at high temperatures for a long period (30-45 minutes) reduced their rate of growth compared to rats fed the same volume of raw milk, with signs of malnutrition occurring over the experimental periods. Rats fed milk held at high temperatures for short periods (1 minute) resulted in no detrimental growth outcomes compared to rats fed raw milk. The authors also supplemented 30-45 minute pasteurised milk and evaporated/condensed milks with calcium lactate, calcium phosphate, calcium glycerophosphate or the washings from pasteurisation. The feeding of these milks to rats resulted in growth curves similar to those seen with rats fed raw milk.

The findings of Daniels and Loughlin indicate that the specific type of pasteurisation method used on milk can have a significant effect on the quality of the final product. However the limited quality of the study's methodology, coupled with the poor reporting of results, means that a broader interpretation of the results cannot be made. This study is not suitable for determining an association between raw/pasteurised milk consumption and human growth and development.

5.1.2 Krauss et al. (1933)

This study on rats by Krauss *et al*. contains several sub-components that assessed different physiological outcomes including growth, calcium/phosphorus status, and the deficiency status associated with several vitamins. The following discussion will focus on the growth component of the study, with other components discussed later in this report.

The study design and methodology used by Krauss *et al*. was of sound quality, although as a study on rats, its applicability to human growth and development is limited. The feeding of either raw milk or pasteurised milk to 12 pairs of rats (in the pairs, one received raw milk, the other pasteurised milk) over 12 weeks showed that the rats given raw milk had a greater gain in weight than the rats given pasteurised milk. However the authors reported that this was a crude aggregated result, and that when the data were assessed according to the pairing of the rats, the differences in growth were found to be statistically non-significant (the level of significance applied to the data was not reported).

5.1.3 Taylor (1931)

The study by Taylor was conducted on school children using the compulsory milk consumption programs that operated in British schools during the early part of the 20^{th} Century. The results of this study indicate that after a four month period, the consumption of raw (unpasteurised) milk made no appreciable difference to weight or height gains compared to the same volume of consumption of pasteurised milk. The subjects consuming either type of milk experienced a greater increase in weight and height compared to those without milk supplementation in their diets.

The findings of this study were challenged in an editorial letter by Fisher and Bartlett (1931). This letter stated that raw milk consumption produced greater gains in height compared to pasteurised milk consumption, as greater gains were reported in 11 of the 14 age categories. However it is not certain whether this is an accurate criticism, as neither Fisher and Bartlett nor Taylor have commented on the statistical analyses that they applied to the results obtained. It is possible that the gains reported on height are not significant, and can be accounted for by other sources of statistical variation. Also, it is possible that other dietary factors could have explained the observed gains in weight and height, as there appears to have been no monitoring of confounding dietary variables.

Due to the above methodological issues, FSANZ considers this study to be unsuitable for making comparisons on the growth and development associated with raw versus pasteurised milk.

5.1.4 Conclusion on the association between raw milk consumption and growth and development

Two of the three studies listed above use a rat model, which is a poor model for human growth and development, and the remaining human trial has substantial methodological problems that reduce the viability of the reported results. This evidence therefore does not provide sufficient justification for further investigation of the potential association between raw milk consumption and growth and development.

5.2 Cardiovascular disease

Submitter comments were received stating that the heat modification of milk proteins causes cardiovascular disease. These comments cited four articles by J.C. Annand, (Annand, 1967; Annand, 1971; Annand, 1972; Annand, 1986).

None of the four articles cited are original research; each article is a review or editorial commenting on the literature available at the time. Annand does however make reference in all of these articles to his own research undertaken in the late 1950s and early 1960s on milk proteins and cardiovascular disease. This research was a series of ecological studies based in the United Kingdom, which assessed cardiovascular disease mortality data (mortality from angina pectoris, coronary heart disease, or cerebral embolism and thrombosis) against the year that pasteurisation was introduced across regional areas. Annand states that these mortality rates were lower in the four years preceding the introduction of pasteurisation in the United Kingdom, and increased by 30-100% in the four years following pasteurisation. It is not clear if this is a real trend though, as Annand also recognises that heart disease classifications changed during the period that pasteurisation was introduced (Annand, 1967).

The four review articles were used by Annand to elaborate his hypothesis that the heating of milk proteins was responsible for increases observed in cardiovascular disease since the 1920s. The first three articles (Annand, 1967; Annand, 1971; Annand, 1972) include consideration of the diets of other ethnic groups, or national nutrition data of nations other than the United Kingdom, to illustrate correlations between milk protein versus milk fat intake and cardiovascular disease. The last article (Annand, 1986) expands the hypothesis with information on milk protein antibody levels and ischaemic heart disease outcomes.

Although Annand presents a case for a relationship between the heating of milk proteins and cardiovascular disease, there were many other competing theories on the causes of cardiovascular disease at the time of his articles. The currently accepted position on the aetiology of cardiovascular disease is that it is a multi-factorial condition, with diet as one contributor only. The fat profile of the diet, particularly the intake of saturated fat, is considered the most important dietary determinant for cardiovascular disease (Hooper *et al*., 2000).

5.2.1 Conclusion on the association between raw milk consumption and cardiovascular disease

The four articles by Annand present a hypothesis on the causes of cardiovascular disease mortality without further supporting experimental evidence. As such, this information does not provide a sufficient basis for further investigation of the potential association between raw milk consumption and cardiovascular disease.

5.3 Nutrient profile of milk - Vitamin C content

Submissions to the Discussion Paper for Proposal P1007 cite two studies (Hess, 1916; Woessner *et al*., 1939) as evidence that the pasteurisation process decreases the vitamin C content of milk, and thus protects against the development of scurvy. A summary of the study characteristics and their overall quality is presented in Table A3 of the Appendix to this report.

Three reviews (Hess, 1917; Hess, 1932) were also mentioned in submissions as supporting this relationship. A study by Krauss *et al*. (1933) was mentioned in submissions, however this study has been treated as a review article, as it only comments on and does not assess the vitamin C content of milk.

5.3.1 Hess (1916)

This early study is a report on eleven clinical cases with infantile scurvy, and pasteurised milk feeding regimes used at the time to treat these cases [1]. However, this study does not compare raw versus unpasteurised milk feeding regimes, rather it involves various modifications to the pasteurised milk diet of the infant cases. Therefore this study is inadequate for determining whether pasteurised milk is more likely to contribute to the development of scurvy than raw milk.

5.3.2 Woessner et al. (1939)

The study by Woessner *et al*. is an analytical assessment of the vitamin C content of different milks. The aim of the study was to compare raw milk to commercial milk supplies of the time rather than to make comparisons with pasteurised milk, although both raw and pasteurised milk samples were included in the study.

The vitamin C contents of the milk samples indicated that the pasteurised milks had lower vitamin C contents than the raw milk samples. The simple and standard approach of the study by Woessner *et al.* lends credibility to these results. It is likely that the trends identified are an accurate representation of vitamin C contents of different milks available in 1939. It is not certain how applicable these results are to modern milk supplies, as Woessner *et al.* did not provide any detail on the pasteurisation processes used for their samples.

[1] Readers should note that while it was considered acceptable in the early 20th Century to exclusively feed infants with pasteurised milk, modern infant feeding guidelines recommend against the feeding of cow's milk (of any type) to infants, as it is an unsuitable form of nutrition for this age group (NHMRC, 2003).

5.3.3 Review articles

The causes of scurvy and its relationship to vitamin C intake were in the process of being identified during the early part of the 20th Century. This is evident in the two review articles by Hess at early and later time periods (Hess, 1917; Hess, 1932). In the first review, Hess extends his hypotheses developed previously on infants (Hess, 1916). However, Hess' second review follows a period of research on scurvy whereby the condition had been recognised as a vitamin C deficiency state. As a result, Hess provides two conclusions on the pasteurisation of milk:

- Pasteurisation decreases the vitamin C content of milk; and
- The final level of vitamin C in the milk depends on the feeding practices of the cattle source.

The two reviews by Hess show a shift in scientific and medical thinking away from categorising foods such as pasteurised milk as a cause of scurvy, to one that recognises scurvy as being dependent on the vitamin C intake across the whole diet. From a historical perspective, the work by Hess is regarded as important for showing that scurvy could be prevented not by focusing on single foods, but by using foods high in vitamin C to complement those with low levels (Rajakumar, 2001).

The change in thinking that occurred towards scurvy is reflected in the article by Krauss *et al*. (1933), who restate the conclusions of Hess (1932) and also indicate that even raw milk is unlikely to provide enough vitamin C by itself in the diet.

5.3.4 Conclusion on the pasteurisation of milk and vitamin C content

The studies and reviews presented in submissions indicate that pasteurised milk has a reduced vitamin C content compared to unpasteurised milk. Current analytical data (FSANZ, 2006) also shows that pasteurised cow's milk has a low vitamin C content at 1 mg/100mL (current data is unavailable for unpasteurised milk).

However as discussed above, scurvy is the result of a deficiency in vitamin C intake across the whole diet, not the result of a low vitamin C content in one food, such as milk. In the context of current Australian and New Zealand diets, milk is not a major contributor to vitamin C intake, with other foods (e.g. fruits and vegetables) acting as more important sources of this nutrient (McLennan and Podger, 1998).

FSANZ is therefore of the view that pasteurisation decreases the vitamin C content of milk, resulting in pasteurised milk having a lower vitamin C content than unpasteurised milk. However, it is inappropriate to use the studies mentioned above to demonstrate that raw milk will protect against scurvy, or that the reduced vitamin C content of pasteurised milk makes this food less nutritious. The reduced vitamin C content of pasteurised milk does not pose a risk to Australian and New Zealand dietary intakes, and so further investigation of this issue is not warranted.

5.4 Nutrient profile of milk - vitamin A, B vitamins, vitamin D, and iodine

Submitters have commented that pasteurisation processes reduce the levels of a number of nutrients in milk, including vitamin A, vitamin D, some B vitamins, and iodine. The evidence cited in support of these comments were two studies by Krauss *et al*. (1933) and Wheeler *et al*. (1983), and a review by Lewis (1935). A summary of the characteristics and quality of the two studies is provided in Table A4 of the Appendix to this report.

5.4.1 Krauss et al. (1933)

The study design and methodology used by Krauss *et al*. was determined to be of sound quality. Each vitamin under assessment (vitamin A, vitamin B_1, folate, and vitamin D) had its own separate experiment, although these experiments were all carried out in a similar manner. Two groups of rats (n for each group = 5-11) were fed a diet deficient in the vitamin under scrutiny. Each group was then provided with three different levels of milk between 1-10 mL/day depending on the deficiency diet used, with one group fed pasteurised milk, and the other unpasteurised milk. Weights and clinical symptoms of deficiency were measured over eight weeks.

The results of the four experiments showed that both groups of rats lost weight and developed deficiency symptoms in a similar manner. In the case of the vitamin A, vitamin D and folate experiments, the feeding of milk produced the same correction in weight and clinical symptoms regardless of its pasteurisation status. However, in the vitamin B_1 experiment, unpasteurised milk was able to corrected all clinical symptoms of deficiency (polyneuritis) and improve weight, while a deficiency state continued with the feeding of pasteurised milk. The authors concluded that of the vitamins assessed in milk, pasteurisation decreases the vitamin B_1 content only.

5.4.2 Wheeler et al. (1983)

This Australian study by Wheeler *et al*. was conducted to determine if pasteurisation was an effective method for reducing iodine levels in milk. Iodine was considered a contaminant of milk at the time of the study's publication (1983), which occurred from the then practice of using iodophors as sanitising agents in milk production. Reducing the iodine content of milk was therefore an important line of research at that time. These chemical agents have now been replaced by other disinfectants, subsequently resulting in a decreased iodine content in Australian and New Zealand milk supplies (Thomson *et al*., 1997).

The study by Wheeler *et al*. is an analytical comparison of the iodine concentrations of pasteurised versus unpasteurised milks. The study was well designed and controlled, using two different analytical methods (chemical and electrode) to increase the reliability of the results. All samples were obtained unpasteurised and iodophor-free, then separated into two groups with one deliberately contaminated with iodine, and the two groups further separated into groups for pasteurisation / no pasteurisation. An analysis of variance on the results for both whey and casein samples showed no significant difference ($p>0.05$) in the iodine concentration of pasteurised versus unpasteurised milk. The authors therefore concluded that pasteurisation cannot be relied upon to decrease the iodine concentration of manufactured dairy products.

5.4.3 Review article – Lewis (1935)

A review article by Lewis was published as a commentary on the role of vitamins and nutrition during pregnancy, as it was known at the time. There is little mention in this article of how pasteurisation can affect the level of vitamins in milk, although it was stated that information up to 1935 indicated that pasteurisation may result in a decrease in the B vitamin content of milk.

5.4.4 Conclusion on pasteurisation and the levels of vitamin A, B vitamins, vitamin D and iodine in milk

The evidence presented by submitters on the influence of pasteurisation on vitamin A, B vitamins, vitamin D and iodine contents of milk may have been applied out of context, as this evidence demonstrates that pasteurisation has little or no influence on the levels in milk of the above mentioned vitamins and minerals. There is some indication from Krauss *et al*. (1933) that pasteurisation may decrease vitamin B_1 content slightly. However milk is not considered to be a major source of this nutrient in the Australian and New Zealand diets (McLennan and Podger, 1998).

FSANZ is therefore of the view that the above evidence is insufficient to warrant further investigation of this issue.

5.5 Availability of folate and calcium from milk

Comments from some submitters indicated that there is evidence showing that pasteurisation alters the calcium and folate in milk, so that their intestinal absorption is reduced. Three studies were cited in support of these comments; one study on folate (Gregory, III, 1982), and two studies on calcium (Kramer *et al*., 1928; Krauss *et al*., 1933). Details on the study characteristics and their quality can be found in Table A5 of the Appendix to this report.

5.5.1 Gregory III (1982)

This study is an analytical assessment of the folate-binding properties of milk, using both radio-isotope labelling and chromatography techniques. Specifically, the study investigated changes in folate-binding protein (FBP) and how much folate was bound to this component of unpasteurised versus pasteurised milk (and pasteurised whey protein). The study was of a sound design that was capable of assessing these changes.

The chromatography results from this study show that the levels of FBP were similar between the unpasteurised and pasteurised milk samples, although the levels of FBP were lower in the pasteurised whey protein samples. The radio-isotope results show that pasteurisation decreased the binding of folate to FBP, but for milk samples at pH levels of 9.3 only, and not pH 7.0.

The authors concluded from these results that pasteurisation alters the structural and functional characteristics of FBP. However, they were unable to hypothesise from these results whether the changes to FBP would affect the body's uptake of folate from pasteurised milk.

5.5.2 Kramer (1928)

The aim of this study was to determine if processing techniques affected the availability of calcium and phosphorus from milk. The study was conducted as an assessment of calcium and phosphorus balance using one group of children and two groups of adult women, although comparisons between unpasteurised (fresh) milk and pasteurised milk were conducted only on the second adult group. The results for the second adult group show that in these subjects, the pasteurised milk consumption resulted in a lower calcium and phosphorus balance than unpasteurised milk (statistical significance not reported).

It is difficult to determine if the changes observed by Kramer are related to the pasteurisation of milk. While there was strict dietary control over subjects, the authors did not provide baseline data on the calcium and phosphorus intakes of subjects. It is thus unclear if the results relate to dietary changes or the health of subjects. Also, all subjects were recruited as volunteers from institutions, and so an effect from selection bias cannot be ruled out. The very low subject numbers used in the study cast further doubt over the validity of the results. Because of these problems, the findings of Kramer (1928) are unsuitable for drawing a conclusion on relationship between pasteurisation and the availability of calcium.

5.5.3 Krauss et al. (1933)

The study design and methodology used by Krauss *et al*. was determined to be of sound quality. The calcium/phosphorus component of this study was a secondary experiment conducted on rats from a previous experiment on iron status following the introduction of raw versus pasteurised milk into their diets. The experiment was therefore not designed specifically to control for calcium and phosphorus status, however the procedures carried out were still adequate for this purpose.

Following the death of the rats from the iron status trial, their femurs were removed and ashed to provide a reference for ash, calcium and phosphorus proportions. The remaining body was then dried and ashed for a final determination of the calcium and phosphorus levels. The results show no differences in these levels between the raw and pasteurised milk groups. The authors concluded from these results that rats were utilising calcium and phosphorus from pasteurised milk as effectively as calcium and phosphorus from raw milk.

5.5.4 Conclusion on the availability of folate and calcium

For both folate and calcium, there is only a limited amount of evidence cited in respect to their availability from pasteurised versus unpasteurised milk. The single study cited by submitters as an indication that pasteurisation reduces the availability of folate from milk actually does not demonstrate such an effect, only that the protein carrier for folate may be affected by pasteurisation. FSANZ is aware of other more recent studies that have directly analysed the bioavailability of folate in milk (de Jong *et al*., 2005; Wigertz *et al*., 1996), which confirm the effects on folate binding protein but also show that the availability of folate itself is unaffected by pasteurisation.

In respect to calcium availability, the cited human study is insufficiently designed to show an effect, and contains substantial methodological limitations. The other cited rat study shows no effect on the availability of calcium from milk following pasteurisation.

Due to the lack of support from the above studies, FSANZ has determined that no further investigation of this issue is required.

6. Reference List

1. Alm, J.S., Swartz, J., Lilja, G., Scheynius, A. and Pershagen, G. (1999) Atopy in children of families with an anthroposophic lifestyle. *Lancet* 353(9163):1485-1488.
2. Annand, J.C. (1967) Hypothesis: heated milk protein and thrombosis. *J Atheroscler Res* 7:797-801.
3. Annand, J.C. (1971) The case against heated milk protein. *Atherosclerosis* 13(1):137-139.
4. Annand, J.C. (1972) Further evidence in the case against heated milk protein. *Atherosclerosis* 15(1):129-133.
5. Annand, J.C. (1986) Denatured bovine immunoglobulin pathogenic in atherosclerosis. *Atherosclerosis* 59(3):347-351.
6. Barnes, M., Cullinan, P., Athanasaki, P., MacNeill, S., Hole, A.M., Harris, J., Kalogeraki, S., Chatzinikolaou, M., Drakonakis, N., Bibaki-Liakou, V., Newman Taylor, A.J. and Bibakis, I. (2001) Crete: does farming explain urban and rural differences in atopy? *Clin Exp Allergy* 31(12):1822-1828.
7. Blaser, M.J., Sazie, E. and Williams, L.P., Jr. (1987) The influence of immunity on raw milk--associated Campylobacter infection. *JAMA* 257(1):43-46.
8. Daniels, A.L. and Loughlin, R. (1920) A deficiency in heat-treated milks. *J Biol Chem* 44:381-397.
9. de Jong, R.J., Verwei, M., West, C.E., van, V.T., Siebelink, E., van den, B.H. and Castenmiller, J.J. (2005) Bioavailability of folic acid from fortified pasteurised and UHT-treated milk in humans. *Eur J Clin Nutr* 59(8):906-913.
10. European Commission (2004) *Infant and young child feeding: standard recommendations for the European Union*. European Commission - Directorate Public Health and Risk Assessment.
11. Fisher, R.A. and Bartlett, S. (1931) Pasteurised and Raw Milk. *Nature* 127(3207):591-592.
12. FSANZ. (2006) *NUTTAB 2006: Australian Food Composition Tables*. FSANZ, Canberra.
13. Gregory, J.F., III. (1982) Denaturation of the folacin-binding protein in pasteurized milk products. *J Nutr* 112(7):1329-1338.
14. Harris, L.J. (1935) *Vitamins in Theory and Practice*. University Press, Cambridge, p224.
15. Hess, A.F. (1916) Infantile Scurvy III: Its Inflence on Growth. *Arch Pediatr Adolesc Med* XII(2):152-165.
16. Hess, A.F. (1917) Infantile Scurvy V: A Study of its Pathogenesis. *Am J Dis Child* 14(5):337-353.
17. Hess, A.F. (1932) Recent Advances in knowledge of scurvy and the antiscorbutic vitamin. *JAMA* :

18. Hollis, B.W., Roos, B.A., Draper, H.H. and Lambert, P.W. (1981) Vitamin D and its metabolites in human and bovine milk. *J Nutr* 111(7):1240-1248.

19. Hooper, L., Summerbell, C.D., Higgins, J.P.T., Thompson, R.L., Clements, G., Capps, N., Davey Smith, G., Riemersma, R. and Ebrahim, S. (2000) *Reduced or modified dietary fat for preventing cardiovascular disease (review)*. Cochrane Database of Systematic Reviews.

20. Jordan, E.O. (1938) *Textbook of General Bacteriology*. 12th ed, W.B.Saunders Co., p691.

21. Kramer, M., Latzke, E. and Shaw, M.M. (1928) A comparison of raw, pasteurized, evaporated, and dried milks as sources of calcium and phosphorus for the human subject. *J Biol Chem* 79(1):283-295.

22. Krauss, W.E., Erb, J.H. and Washburn, R.G. (1933) Studies on the nutritive value of milk II: the effect of pasteurization on some of the nutritive properties of milk. *Ohio Agricultural Experiment Station Bulletin* 518:8.

23. Lewis, L.R. (1935) The relation of the vitamins to obstetrics. *Am J Obs Gynecology* 29(5):759.

24. McLennan, W. and Podger, A. (1998) *National Nutrition Survey. Nutrient intakes and physical measurements.* Australian Bureau of Statistics, Canberra.

25. Ministry of Health. (2003) *Food and Nutrition Guidelines for Healthy Adults: A Background Paper*. New Zealand Ministry of Health, Wellington.

26. NHMRC. (2003) *Food for Health: Dietary Guidelines for Children and Adolescents in Australia, incorporating the Infant Feeding Guidelines for Health Workers*. Commonwealth Department of Health and Ageing, Canberra, p45-66.

27. Perkin, M.R. (2007) Unpasteurized milk: health or hazard? *Clin Exp Allergy* 37(5):627-630.

28. Perkin, M.R. and Strachan, D.P. (2006) Which aspects of the farming lifestyle explain the inverse association with childhood allergy? *J Allergy Clin Immunol.* 117(6):1374-1381.

29. Pettifor, J.M., Stein, H., Herman, A., Ross, F.P., Blumenfeld, T. and Moodley, G.P. (1986) Mineral homeostasis in very low birth weight infants fed either own mother's milk or pooled pasteurized preterm milk. *J Pediatr Gastroenterol Nutr* 5(2):248-253.

30. Pottenger Jr, F.M., Pottenger, E. and Pottenger, R.T. (1983) *Pottenger's Cats: A Study in Nutrition*. 2nd ed, Price-Pottenger Nutrition Foundation, California.

31. Radon, K., Windstetter, D., Eckart, J., Dressel, H., Leitritz, L., Reichert, J., Schmid, M., Praml, G., Schosser, M., von, M.E. and Nowak, D. (2004) Farming exposure in childhood, exposure to markers of infections and the development of atopy in rural subjects. *Clin Exp Allergy* 34(8):1178-1183.

32. Rajakumar, K. (2001) Infantile scurvy: a historical perspective. *Pediatrics* 108(4):E76.

33. Remes, S.T., Iivanainen, K., Koskela, H. and Pekkanen, J. (2003) Which factors explain the lower prevalence of atopy amongst farmers' children? *Clin Exp Allergy* 33(4):427-434.

34. Riedler, J., Braun-Fahrlander, C., Eder, W., Schreuer, M., Waser, M., Maisch, S., Carr, D., Schierl, R., Nowak, D. and von, M.E. (2001) Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 358(9288):1129-1133.

35. Schmidt-Nielsen, S. and Schmidt-Nielson. (1930) [Untitled]. *Kgl.Norske Videnskab.Selsk.Forhandl.* 1:126-128.

36. Scott, E. and Lowell, A.E. (1931) Ohio Tests Prove Natural Milk is Best. *Jersey Bulletin* 50:210-211.

37. Strachan, D.P. (1989) Hay fever, hygiene, and household size. *BMJ* 299(6710):1259-1260.

38. Taylor, J. (1931) Milk Tests in Lanarkshire Schools. *Nature* 127(3203):466.

39. Thomson, C.D., Colls, A.J., Conaglen, J.V., Macormack, M., Stiles, M. and Mann, J. (1997) Iodine status of New Zealand residents as assessed by urinary iodide excretion and thyroid hormones. *Br.J.Nutr.* 78(6):901-912.

40. Waser, M., Michels, K.B., Bieli, C., Floistrup, H., Pershagen, G., von, M.E., Ege, M., Riedler, J., Schram-Bijkerk, D., Brunekreef, B., van, H.M., Lauener, R. and Braun-Fahrlander, C. (2007)

Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe. *Clin Exp Allergy* 37(5):661-670.

41. Wheeler, S.M., Fleet, G.H. and Ashley, R.J. (1983) Effect of processing upon concentration and distribution of natural and iodophor-derived iodine in milk. *J Dairy Sci* 66(2):187-195.

42. Wickens, K., Lane, J.M., Fitzharris, P., Siebers, R., Riley, G., Douwes, J., Smith, T. and Crane, J. (2002) Farm residence and exposures and the risk of allergic diseases in New Zealand children. *Allergy* 57(12):1171-1179.

43. Wigertz, K., Hansen, I., Hoier-Madsen, M., Holm, J. and Jagerstad, M. (1996) Effect of milk processing on the concentration of folate-binding protein (FBP), folate-binding capacity and retention of 5-methyltetrahydrofolate. *Int J Food Sci Nutr* 47(4):315-322.

44. Woessner, W.W., Elvehjem, C.A. and Schuette, H.A. (1939) The Determination of Ascorbic Acid in Commercial Milks. *J Nutr* 18(6):619-626.

45. Wulzen, R. and Bahrs, A.M. (1941) Effects of milk diets on guinea pigs. *Am J Physiol* 133:500.

Appendix

Design and Quality of Identified Studies

Table A1: Studies on raw milk and allergy sensitisation

Study	Hypothesis	Design and location	Endpoint measurements	Subject groupings	Assessment and measurement	Bias	Confounders / adjustments	Overall quality
Barnes et al. (2001)	Theory that rates of atopy are lower amongst children from rural versus urban communities. To determine if the lower atopy is explained by contact with farm animals or unpasteurised milk in early childhood.	Case-control study. 997 parents of children aged 11-19 completed a questionnaire on atopy (72% response rate). 930 of these children (93%) received skin prick testing and provided blood samples. Conducted on the island of Crete.	Atopy as measured by skin prick testing. Blood samples were used to determine the hepatitis A status of subjects.	The 929 skin prick subjects were grouped into rural (n=418) and urban (n=512) children for demographic analysis. No significant ($p<0.05$) differences in the two groups were noted except for education levels of parents. Regression analysis was conducted using the farm animal contact and unpasteurised milk consumption status of subjects.	Qualitative: Exposure variables in the questionnaire were adopted from the ISAAC protocol. Additional variables (including unpasteurised milk) were collected in this questionnaire, although the parameters for these variables were not described. The exposures were for the first five years of life Quantitative: A positive skin prick test was defined as ≥3mm mean response.	Inclusion and exclusion rates were well documented. There is the potential for recall bias, due to dietary information over six years old.	A core model was used for the regression analysis. The model included age, sex, parental allergy, number of siblings and length of rural location.	Category: **Observational** A well-designed and executed study. The similarities in the demographics of the rural and urban populations, along with a moderately sized sample population assists in the attainment of valid results. A minor concern is the lack of reporting on the dietary protocol, although this is not likely to be of significance as multiple dietary interactions were not assessed. The potential for recall bias is also another minor concern with this study design.
Perkin and Strachan (2006)	Hypothesis that farming lifestyle is associated with improved allergy tolerance. Designed to test whether barn exposure, animal contact, or unpasteurised milk exposure improves allergy outcomes.	Case-control study. Was the second stage of the broader Study of Asthma and Allergy in Shropshire 7226 questionnaires sent to children in 73 schools of same region. Response rate of 66%. 879 subjects agreed to skin prick testing. UK study.	From questionnaire to parents (on previous childhood exposures): asthma, seasonal rhinitis, eczema. Atopy: positive skin prick test	4767 subjects completed questionnaire. Subjects were grouped into children whose parents lived on farm (n=535), farm labourer's children (n=281), and control children (n=3951). Age of children was not reported.	Dietary assessment by food frequency questionnaire. Unpasteurised milk consumption was an explicit component of the survey. Assessment was for prior dietary habits. A positive skin prick test was defined as ≥3mm mean response.	Inclusion and exclusion rates were well documented. Recall bias may be an issue, as it is unknown how long into the past the survey was assessing.	Adjustments made for known allergy confounders: age, sex, breastfeeding, family history of atopy, and sibling numbers. Second adjustment for farm lifestyle variables.	Category: **Observational.** A well designed study with some minor flaws in respect to recall bias and the need to use second adjustments to highlight unpasteurised milk results.

Study	Hypothesis	Design and location	Endpoint measurements	Subject groupings	Assessment and measurement	Bias	Confounders / adjustments	Overall quality
Radon et al. (2004)	Hypothesis that farming lifestyle is associated with improved allergy tolerance. Designed to test the association between farming-related risk factors and seropositivity to *T.gondii* and *H.pylori*.	Case-control study. Was nested in a larger study on asthma and allergies in rural environments. 3112 questionnaires received after 6 weeks (68.6% response rate) from 18-44 year olds. North German study.	Prevalence of *T.gondii* and *H.pylori* infection and seropositivity. Association between farming environment, markers of infection, and atopy.	930 subjects were randomly selected from those returning questionnaires for clinical examinations. 520 of these agreed to venous blood sampling. Subjects were divided into those with atopy (n=230) and controls (n=91). For multivariate analysis subjects were assessed on raw milk consumption, age of first visit to animal houses, and markers of infection.	The study reports on consumption of 'raw uncooked farm milk' amongst its assessment of farming related factors, but does not detail how these factors were collected. IgE assays were conducted for atopy assessments. IgG assays were used for seropositivity assessments. Exposure variables were for the period less than 6 years of age.	Inclusion and exclusion rates were well documented. Researchers were blinded to the atopy status of the subjects.	Adjustments made for age, sex, smoking, living on farm at time of study, family history of atopy, and sibling numbers.	Category: **Observational.** The study has a sound design, however the study was not primarily targeted at the association of unpasteurised milk. Dietary assessments were not reported, and the main concern was with the *T.gondii* and *H.pylori* infection prevalence. The lack of reporting on the dietary component makes it difficult to determine what is meant by 'farm milk'.
Remes et al. (2003)	Theory that differences in environmental factors explain the different rates of atopy between farmers' versus non-farmers' children	Case-control study. 924 children aged 6-15 years were sent questionnaires. Subjects enrolled as farming children (n=462) or non-farming children matched from the same community (n=462). 765 returned questionnaires (82.8% response rate). 714 children took part in further skin prick testing (77.3% participation rate). Study conducted in 17 Finnish towns.	Atopy as determined by skin prick testing to six common allergens (birch, timothy grass and mugwort pollens, cat and dog danders, and house dust mite).	Subjects were compared on the basis of farming versus non-farming groups. For multivariate analysis, subjects were broken down according to their assessed characteristics, including farm milk consumption and contact with livestock.	Dietary assessment using PARSIFAL and FINRICK questions. A food frequency questionnaire was not used. A positive skin prick test was defined as ≥3mm mean response. Tests were performed in accordance with ISAAC study protocols using trained nursing staff, and included positive and negative control tests. Exposure variables were during infancy.	Inclusion and exclusion rates, along with attrition were well documented. There may be recall bias due to dietary information over 5 years old.	Adjustments were made for sex, age, parental hayfever, parental education, older siblings, daycare in first year of life, and current farming.	Category: **Observational.** A well designed study that is comprehensive in its assessment of possible exposure variables. There are some flaws regarding the thoroughness of the dietary assessments, although the authors have been clear on the processes used. The potential for recall bias means that some of the results on exposures during infancy may be inaccurate. However, these minor issues are unlikely to affect the overall validity of the results.

Study	Hypothesis	Design and location	Endpoint measurements	Subject groupings	Assessment and measurement	Bias	Confounders / adjustments	Overall quality
Riedler et al. (2001)	Hypothesis that the exposure to a farming environment influences the protection conferred on asthma, hayfever and allergic sensitisation.	Case-control study. 3504 children of school grades 1-6 (mean age = 9.4 years) were invited to the survey. 2618 children participated in the questionnaire (75% response rate). 1406 agreed to further blood sampling (40% participation rate). Authors report that complete data was available only for 812 children. Conducted in Austria, Germany and Switzerland.	From questionnaire to parents (on previous childhood exposures): diagnosis of hayfever, asthma, current wheeze. Atopic sensitisation was measured by serum IgE for six aero-allergens and six food allergens.	Subjects were compared on the basis of being part of a farming (n=319) versus non-farming (n=493) family. For analysis against endpoints, subjects were broken down according to a wide range of exposure variables, including farm milk consumption and time visiting stables during infancy.	Questions on allergy symptoms (in previous 12 months) were based on ISAAC protocols. Dietary information was collected as part of supplementary interview by trained staff. Serum IgE assessed via assay, with atopic sensitisation determined as at least one result of ≥3.5 kU/L for the six aeroallergens. Exposure variables were during infancy.	There are questions over the exclusion rates, as the authors have not explained the missing data that reduced the sample to 812. There may be recall bias due to dietary information over 5 years old (noted by the authors).	Adjustments were made for age, sex, parental education, family history of hatfever, older siblings, and farming status.	Category: **Observational.** There are some problems with this study that could affect the viability of the results obtained. The response/participation rates for the study are very low, and the authors have not fully explained the exclusions that occurred as part of their study. It is therefore possible that there is selection bias that could skew the results in a particular direction. The possibility of recall bias also limits the viability of the results obtained.
Waser et al. (2007)	Aimed to specifically investigate whether the consumption of self or farm produced products (especially milk) is associated with a lower prevalence of asthma, allergic diseases and atopic sensitisation.	Case-control study. Data was taken from the PARSIFAL study. Data for 15137 children aged 5-13 years. Of this number 8788 consented to blood sampling, and 4854 children gave a blood sample. 4049 of these actually gave a sample (83.4% participation rate), and 3979 with enough volume for IgE assays. Conducted in Australia, Germany, Switzerland, Netherlands and Sweden.	From questionnaire to parents (on previous childhood exposures): diagnosis of asthma, hayfever, eczema and rhino-conjunctivits. Also self report of current wheezing. Atopic sensitisation was measured by IgE assay of 9 aero-allergens and 6 food allergens.	Subjects were compared on the basis farming children (n=2823) versus a non-farming reference (n=5440), and children attending Rudolf Steiner Schools (n=4606) versus children attending other schools (n=2024). Subject's dietary characteristics (including 'farm milk' consumption) formed the basis of multivariate analyses.	A food frequency questionnaire was used in the PARSIFAL study for current and infancy dietary exposures. Frequency data was verified through the use of 24-hour recalls. The prevalence of diseases and conditions was assessed in the questionnaire using ISAAC protocols. Serum IgE assessed via assay, with atopic sensitisation determined as ≥0.35 kU/L.	Inclusion and exclusion rates were well documented.	Adjustments were made for study groupings, nationality, sex, age, parental history of asthma or hayfever, parental education, smoking during pregnancy, environmental smoking, older siblings, exclusive breastfeeding >4 months, BMI and food avoidance.	Category: **Observational.** The study has a sound design, and has large enough samples to generate accurate results. The authors have also documented their assessment procedures and recruitment protocols in detail, further lending confidence to the viability of the results generated. The authors do acknowledge, however, that confirmation of the status of raw milk intake could not be made. This was considered a problem by the authors, as parents within the assessed countries are advised against feeding raw milk to children, and so they may have reported this variable incorrectly.

Study	Hypothesis	Design and location	Endpoint measurements	Subject groupings	Assessment and measurement	Bias	Confounders / adjustments	Overall quality
Wickens et al. (2002)	Aim was to assess whether the protective effect of a farming lifestyle against allergic diseases applies to New Zealand environments. A secondary aim of the study was to determine what farming-related factors contribute to allergy outcomes.	Case-control study. 605 7-10 year old school children were included as subjects. 111 were excluded as siblings attending the same school. 494 subjects were therefore assessed. 293 questionnaires, 286 dust samples, and 275 skin prick tests were completed (60%, 58%, 56% response rates respectively). New Zealand study.	From questionnaire to parents (on previous childhood exposures): hayfever, asthma, current wheeze, eczema. Atopy measured by a positive skin prick tests to common allergens (*D.farinae, D.pteronyssinus,* mould, cockroach, rye, grass, timothy grass, cat, dog).	The cohort of 293 subjects were grouped into those that currently live on an operational farm (n=95) and non-farm subjects (n=198) for allergy symptoms and skin prick tests. For multivariate analysis, subjects were broken down according to a wide range of exposure variables. Dairy categories included yoghurt 1+ a week (n=225), unpasteurised milk ever (n=38), pasteurised milk 1+ a day (n=192), and cheese 1+ a week (n=200).	The study reports on consumption of 'dairy food consumption' amongst its assessment of farming related factors, but does not detail how these factors were collected. It was mentioned that dietary information was collected for the first 2 years of life and as a current intake. A positive skin prick test was defined as ≥3mm mean response. Standard methods were used for the skin prick test.	Inclusion and exclusion rates were well documented. There may be recall bias due to dietary information over 5 years old. Authors report that there may be selection bias due to low (<50%) response rate from two schools.	Univariate analyses of subject characteristics and allergy symptoms were used for adjustment in the multivariate analysis, along with gender, mother's education level, family history of disease, family size and antibiotic use.	Category: **Observational** This study's design, while directed at assessing farm-related variables, was not primarily aimed at an assessment of unpasteurised milk consumption. The recollection period for exposure variables was lengthy and so may have contributed to recall bias. Low subject response rates may also have produced some inaccuracies in the data. .

Table A2: Studies on raw milk consumption and growth/development

Study	Hypothesis	Design and location	Endpoint measure-ments	Subject groupings	Assessment and measurement	Bias	Confounders / adjustments	Overall quality
Daniels (1928)	A longer length of heating during the pasteurisation of milk decreases its nutritional value compared to shorter periods of heating.	*In vivo* study on rats. 122 rats were assessed in 19 separate dietary regime groups. Some diets consisted of exclusively feeding of: • Pasteurised milk held at high temperatures for either short or long times; • Evaporated milk; or • Condensed milk. The above diets were repeated with added calcium lactate, calcium phosphate, calcium glycerophosphate or washing from pasteurisation. Vitamin restricted diets were also fed to four groups + evaporated or raw milk, or the creams of this milk. United States study.	Weight measured at four week intervals	Each group consisted between 8-10 rats. Rearing characteristics of the rats were not described, nor was their breed or age at commencement of the study periods.	Pasteurisation occurred at temperatures of 114°C for either 1 or 45 minutes (short and long held milks respectively). Evaporated milk was produced by boiling for 10 minutes, then placing in a vacuum at 55°C. Condensed milk was produced by heating to 94°C then dropping to 50°C as the milk condenses.	Authors appear not to have blinded the allocation of rats to groups, or employed randomisation techniques. It is not clear what groups are acting as controls for others, and whether there was heterogeneity between the characteristics of the rats used in the study.	n/a	Category: **Intervention** This study has numerous problems in its design, the most significant being the lack of controls employed for comparisons across different dietary groups. The study is also lacking in detail on important information, such as the physical attributes of the rats, the conditions of their housing during the study, or the quantities of milk provided to each rat. The absence of accurate reporting may, however, be a result of the standards applied to the scientific literature at the time. As a result of the design and reporting flaws, it is doubtful that the results of the study can be considered as accurate or representative of true outcomes.
Krauss et al (1933)	The aim of the study was to compare the growth promoting capacity of raw and pasteurised milks on rats	*In vivo* study conducted on rats. 24 albino rats of 24 weeks of age were divided into 12 pairs based on sex and weight. The rats were exclusively fed raw or pasteurised milk, with the volume given to each pair determined by the poorer consumer. The study was conducted over 12 week. United States study.	Weight.	Within each pair, one rat received raw milk, and the other received pasteurised milk (total of 12 pairs).	Weight was recorded weekly. Haemoglobin levels of the rats were assessed weekly to ensure that anaemia did not develop. All milk was obtained from the same cattle herd, with the cattle maintained in a healthy condition. Pasteurisation was carried out at 62.5°C for 30 minutes.	The authors have taken measures to ensure that the rats are similar in physical attributes, and that all pairs were maintained in the same environmental conditions.	Iron and copper were added to the test milks to prevent the development of anaemia.	Category: **Intervention** A well designed and executed study on rats. The authors have taken measures to maintain homogeneity in the rat subjects and in the environmental and feeding conditions. Although the sample size is small, the controls placed on the study regime provide validity to the results obtained.

Study	Hypothesis	Design and location	Endpoint measure-ments	Subject groupings	Assessment and measurement	Bias	Confounders / adjustments	Overall quality
Taylor (1931)	The addition of milk to the diet of school children affects their growth and height.	Randomised controlled trial. The study is an assessment of data from a trial of compulsory milk consumption in Scottish schools. 20000 children aged 5-13 years were given either a daily ration of unpasteurised or pasteurised milk, or received no milk ration (controls). The milk was in addition to their normal diet. The intervention period lasted for four months. Scottish study.	Weight and height.	The following groups were used: No milk (controls), n=10000; Pasteurised milk, n=5000; Raw milk, n=5000.	The equipment used for measuring weight and height was not reported, or whether it was standardised across school locations. The pasteurisation processes were not reported for the milk used. Local milks were also used across locations, rather than a single source. Dietary controls were not employed in the study, aside from those relating to the intervention itself.	Although randomisation processes were employed, it is not clear if blinding occurred. However, as the milk was administered at the schools, it is unlikely the authors were aware of what subjects received the intervention.	None reported.	Category: **Intervention** There are several gaps within the methodology of this study, most relating to the limited reporting within the study article. One problem that is evident even with the limited reporting is that there was no control or monitoring of the school children's diets. It is therefore difficult to know if the results reflect the dietary intervention or if they are a reflection on the diets outside of the intervention. However, the large subject numbers may assist in reducing inter-subject dietary variability. Overall the findings of this study are to be treated with caution.

Table A3: Studies on pasteurisation and vitamin C content

Study	Hypothesis	Design and location	Endpoint measurements	Subject groupings	Assessment and measurement	Bias	Confounders / adjustments	Overall quality
Hess (1916)	The exclusive consumption of pasteurised milk by infants impairs growth through the development of infantile scurvy.	Case reports. The author presents 11 cases that were examined over the course of the year, with finings presented across different ages (0-12 months) and for differing periods of observation (2-10 months). Cases were of infants admitted to an institution due to severe growth impairment. Cases were from the United States of America.	All findings were presented as weight or length changes with time. Each case represented different feeding scenarios.	N/A	Weighing and length measuring techniques were not reported, although all assessments are likely to be consistent across cases as they were performed within the same institution. Infant diets, both pre- and post-admission were well documented.	As individual case reports, the findings are unlikely to represent all similar cases. There may be some recall bias, as parents may not divulged correct pre-admission feeding details (given that the infants were admitted with malnutrition).	N/A	Category: **Observational** This study has numerous shortcomings, such as its representativeness and lack of reporting on methodologies. The shortcomings reflect the age of the study and the standards that were applied to studies at the time. However, the author has effectively used the cases to illustrate biological mechanisms for scurvy (unknown at the time), and how a whole diet approach can overcome vitamin C deficiencies associated with the then common practice of exclusively feeding (pasteurised) milk to non-breastfed infants.
Woessner (1939)	Not explicitly stated, however the article's discussions indicate a hypothesis that commercial milk supplies have a reduced vitamin C content to that present at the time of milking.	Controlled *in-vitro* trial. Analysis of vitamin C content of milk samples. Randomisation and blinding processes were not described. Control samples were collected from 50 US cows. Breeding and feeding details were described but not age or calving status. Test samples were collected from local (US) dairies. Sample numbers were not reported.	Ascorbic acid and dehydroascorbic acid content of milk samples	Control samples were separated by breed of cattle, and the type of feed obtained by the cattle over the preceding year. Test samples were broken down into unpasteurised milk, pasteurised milk, and milks with modified vitamin D or mineral contents.	Vitamin C analyses were carried out using colourimetric assessment.	It is possible that there may have been some selection bias, as it is unknown what blinding was employed in the study.	The authors report that the analytical techniques were able to account for the presence of riboflavin and turbidity in samples.	Category: **Analytical** The study employs an effective process for assessing the vitamin C content of different milks. However there are significant gaps in the reporting of methodological details, particularly details on the blinding and randomisation processes. Therefore the results obtained may not be entirely valid.

Table A4: Studies on pasteurisation and the contents of vitamin A, B vitamins, vitamin D, and iodine

Study	Hypothesis	Design and location	Endpoint measurements	Subject groupings	Assessment and measurement	Bias	Confounders / adjustments	Overall quality
Krauss et al (1933)	The aim of the study was to compare the growth promoting capacity of raw and pasteurised milks on rats	*In vitro* study conducted on rats. Albino rats were placed on diets deficient in either vitamin A, thiamin, folate or vitamin D for 2-3 weeks. The rats fed either pasteurised or unpasteurised bovine milk (or creams for the vitamin D experiment) for eight weeks, and in three different volumes. The volumes varied from 1-10 mL/day. United States study.	Weight and clinical symptoms of deficiency.	Six groups were assigned to each deficiency (two groups for each milk/cream, with the milk/cream fed in three different volumes). Vitamin A n=41 Thiamin n=46 Folate n=39 Vitamin D n=150	Weight was recorded weekly. Clinical symptoms were monitored by the researchers and reported within the article text.	All milk was obtained from the same cattle herd, with the cattle maintained in a healthy condition. Pasteurisation was carried out at 62.5°C for 30 minutes.	Iron and copper were added to the test milks used in the growth study to prevent the development of anaemia.	Category: **Intervention** A well designed and executed study on rats. The authors have taken measures to maintain homogeneity in the rat subjects and in the environmental and feeding conditions. The controls placed on each separate deficiency intervention provide validity to the results obtained.
Wheeler (1982)	The pasteurisation can be used to decrease the iodine content of milk that occurs following the (then) process of iodophor-based sanitisation.	Analysis of the iodine content of raw milk, raw skimmed milk, pasteurised skim milk, and reconstituted spray dried milk. Milk was obtained from iodophor-free cattle, and divided by contamination with iodophors (500µg/L), or free of contamination. The number of sample replicates used in each analysis ranged between 2-5. Australian study.	Iodine content of milk samples	The milk samples were divided into cream, whey and casein fractions for analysis. Two processing runs were applied to both the adulterated and unadulterated milk, and to the differentially processed samples obtained from each.	Iodine contents were measured by a chemical method (Sandell-Kolthoff method) and an electrode method.	It is possible that there may have been some selection bias, as it is unknown what blinding was employed in the study.	n/a	Category: **Analytical** A well designed and controlled study of the iodine content within milk. Although the study was primarily designed to address issues relating to iodophor sanitisation of milk, its findings are still valid for determining the effects of pasteurisation on milk iodine contents. The authors have identified that the two analytical methods produced significantly different ($p>0.05$) results from each other when applied to some samples. It is therefore possible that the inter-assay variability could have produced results that are not entirely accurate.

Table A5: Studies on pasteurisation and the availability of folate and calcium

Study	Hypothesis	Design and location	Endpoint measurements	Subject groupings	Assessment and measurement	Bias	Confounders / adjustments	Overall quality
Gregory III (1982)	Pasteurisation of milk changes its folate binding activity.	Analysis of the folic acid binding properties of pasteurised and unpasteurised milk. Randomisation and blinding processes were not described. Milk samples were obtained from Florida Dairy Science Dept, with commercial whey samples obtained as a gift.	Two endpoints: Bound, free and total folic acid of samples, following the addition of set quantities of folic acid. Folate binding protein (FBP) concentration of samples.	Both unpasteurised and pasteurised milk were examined, along with a whey protein control. The number of samples used in each analysis was not reported.	Two analytical techniques – liquid scintillation counting of labelled folic acid bound to FBP; and gel-filtrtation chromatographic analysis of FBP.	It is possible that there may have been some selection bias, as it is unknown what blinding was employed in the study.	Authors reported that concentration and pH variations can affect the chromatography. This study therefore kept the pH of samples at 6.3 to minimise these effects.	Category: **Analytical** The study employs a sound and accurate design to determine the extent of folic acid binding in pasteurised versus unpasteurised milk. A limitation of this approach is that it does not determine whether binding properties influence the availability of folic acid. However the design is adequately suited to meet the stated aims of the study.
Kramer (1928)	The processing of milk (drying, evaporating or pasteurisation) changes the availability of calcium and phosphorus compared to raw milk.	Cross-over study. Three healthy cohorts were studied, consisting of 5 children (7-12 years), and two groups of 4 women each (ages not reported). Study periods were for 15, 18 and 24 days respectively. A standard diet was used by each cohort with an additional 625g (children) / 222g (adults) of each test milk (or its equivalent) consumed in periods of three days. No washout periods appear to have been used.	Calcium and phosphorus balance, as measured from food intake against faecal and urinary outputs.	Children: 5 cross-over periods Fresh milk – periods 1, 2 and 5; Dried milk – periods 3 and 4. Adult Group 1: 8 cross-over periods Dried milk – periods 1 and 2; Evaporated milk – periods 5 and 6; Fresh milk – periods 3, 4, 7 and 8. Adult Group 2: 6 cross-over periods Pasteurised milk – periods 1 and 2; Fresh milk – periods 3 and 4; Evaporated or dried milk – periods 5 and 6 (subjects alternated milks over periods).	Weighted food records were kept for each subject. Exercise was monitored but not restricted. Commercial varieties of dried and evaporated milks were used. Fresh and pasteurised milk was obtained from a local dairy. Gravimetric and volumetric methods of analysis were used for both calcium and phosphorus assessments.	There is the potential for selection bias, as subjects were actively recruited from institutional / academic locations. Allocation bias may also be an issue, as subjects were actively assigned to groups by the authors.	Baseline variables were not measured by the authors, except for weight. No blood measurements were taken before or during the study periods.	Category: **Intervention** The control mechanisms employed in the study were rigorous and implemented well. Dietary variables were strictly controlled, and efforts were made to ensure consistent intakes of calcium and phosphorus. However there is likely to be bias due to the recruitment and allocation of subjects. The lack of monitoring makes it difficult to determine if changes in calcium and phosphorus balance are due to the dietary interventions, or as a result of other physiological processes. The bias and limited monitoring combined with the small sample size reduce the applicability of the results obtained.

Study	Hypothesis	Design and location	Endpoint measurements	Subject groupings	Assessment and measurement	Bias	Confounders / adjustments	Overall quality
Krauss et al (1933)	The aim of the study was to compare the growth promoting capacity of raw and pasteurised milks on rats	*In vitro* study conducted on rats. Albino rats from anaemia and growth (n=16) experiments were assessed. The rats were exclusively fed raw or pasteurised milk. The feeding regime was conducted over 24 weeks. United States study.	Calcium and phosphorus content.	16 and 8 pairs were used from the anaemia and studies respectively. These rats had been divided into pairs based on sex and weight.	Upon the death of the anaemia rats, their femurs were removed and assessed for calcium and phosphorus contents. Upon the death of the growth rats, the bodies were dried and ashed. Official analytical methods of the time were used to assess calcium and phosphorus contents.	All milk was obtained from the same cattle herd, with the cattle maintained in a healthy condition. Pasteurisation was carried out at 62.5^{o}C for 30 minutes.	Iron and copper were added to the test milks used in the growth study to prevent the development of anaemia.	Category: **Analytical** A well designed and executed study on rats. The authors have taken measures to maintain homogeneity in the rat subjects and in the environmental and feeding conditions. Although the sample size is small, the controls placed on the study regime provide validity to the results obtained.