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Beneficial nutrients in bovine milk for human health

A review of current knowledge

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Summary

Data from a number of scientific studies in recent years have demonstrated the beneficial effects of bovine milk for human health. For example, calcium and other key nutrients from dairy foods have been shown to reduce the incidence of osteoporosis (Huth *et al*., 2006). The evidence for anti-cancer properties of bovine milk has also been reviewed by Parodi (1994, 1996, 1998, 1999) who concluded that evidence from *in vitro* studies with tumour cell lines and animal models of tumourigenesis indicated that bovine milk contains nutrients with anti-cancer properties. In a more recent review by Gill and Cross (2000), it was concluded that there have been relatively few appropriately designed clinical trials to allow one to conclusively determine the anticancer properties of the nutrients in milk. Milk is also an important source of nutrients required for bone health (Wham and Worsley, 2003) and has positive implications for weight management in humans (Zemel, 2002). Furthermore, it is well recognised that modern society places a greater emphasis on nutritional content and the health benefits of foods. In relation to the dairy industry, this has had a major impact on the development of functional foods or niche products, where milk is a prime source of essential nutrients for general human well-being and health (Peng *et al*., 2006). In a summary of the main findings from the British Nutrition Foundation/Royal Society of Chemistry Conference ‘Functional Foods ’99, Schenker (1999) referred to milk as functional food, due to the presence of a number of immunomodulatory factors. A functional food was defined as “a food having health promoting benefits and/or disease preventing properties over and above its usual nutritional value” (Schenker, 1999). This report encompasses a review of the literature on the contribution of nutrients in bovine milk to human bone health (calcium, phosphorus, magnesium, zinc); mood influences (melatonin, vitamin E, selenium, zinc, omega-3 polyunsaturated fatty acids, lactalbumin); immune health (selenium, zinc, lactoferrin, conjugated linoleic acid, colostrum) and antioxidants (selenium, vitamin E, vitamin C, zinc). The factors that affect the levels of these nutrients in bovine milk (e.g. breed, diet, season) have also been reviewed.

1 Introduction

1.1 Recommended nutrient intake for the human diet

It is well recognised that humans require a balanced diet to maintain good health. The Food Standards Agency (UK) recommend that humans should consume a variety of foods, basing meals on starchy foods, eating at least five portions of fruit and vegetable daily in addition to moderate amounts of meat, fish, milk and dairy products. The Reference Nutrient Intake (RNI) of the various nutrients required for human health is given in Table 1.

Table 1 Reference Nutrient Intake (R) and Lower Reference Nutrient Intake (L) of nutrients by age (years) and sex

		Mineral (mg/day)					Vitamin (mg/day) unless otherwise stated*							
Men		**Ca**	**P**	**Mg**	**K**	**Zn**	**Vit A***	**Th**	**Ri**	**Ni**	**Vit B_2**	**Vit B_{12}***	**Vit D***	**Fol***
19-50	R	700	550	300	3500	9.5	700	1.0	1.3	17	1.4	1.5		200
	L	400	310	190	2000	5.5	300	0.6	0.8	11	1.0	1.0		100
51-64	R	700	550	300	3500	9.5	700	0.9	1.3	16	1.4	1.5		200
	L	400	310	190	2000	5.5	300	0.5	0.8	10	1.0	1.0		100
Women														
19-50	R	700	550	270	3500	7.0	600	0.8	1.0	13	1.2	1.5		200
	L	400	310	150	2000	4.0	250	0.4	0.8	9	0.8	1.0		100
51-64	R	700	550	270	3500	7.0	600	0.8	1.1	12	1.2	1.5		200
	L	400	310	150	2000	4.0	250	0.4	0.8	8	0.8	1.0		100
Children[1]														
0-6 mth													8.5	
7mth-3 yr													7	
Adult[2]														
65+													10	

Th=Thiamin; Ri=Riboflavin; Ni=Niacin; Fol=Folate; *µg/day;

[1] www.nutrition.org.uk/upload/DRVs.pdf (Accessed 26 April 2007)

[2] British Nutrition Foundation (2004)

(Henderson *et al*., 2003)

1.2 Nutrient profile of milk

The average nutrient composition of whole, semi-skimmed and skimmed milk in the UK is given in Table 2. Although the energy and fat content of whole milk is greater than semi-skimmed or skimmed milk, there are marginal differences in the concentration of all other nutrients present.

Table 2 Nutrient content of milk (per 100 ml)

Nutrient	Whole	Semi-Skimmed	Skimmed
Energy (KJ)	282	201	148
Protein (g)	3.4	3.6	3.6
Carbohydrate (g)	4.7	4.8	4.9
of which sugars (g)	4.7	4.8	4.9
Fat (g)	4.0	1.8	0.3
of which saturates	2.6	1.1	0.1
monounsaturates	1.0	0.4	0.1
polyunsaturates	0.1	Trace	Trace
trans fatty acids	0.1	0.1	Trace
Thiamin (mg)	0.03	0.03	0.03
Riboflavin (mg)	0.24	0.25	0.23
Niacin (mg)	0.2	0.1	0.1
Vitamin B_6 (mg)	0.06	0.06	0.06
Vitamin B_{12} (µg)	0.9	0.9	0.8
Vitamin C (mg)	2	2	1
Vitamin D (µg)	Trace	Trace	Trace
Vitamin E (mg)	0.08	0.04	Trace
Sodium (mg)	44	44	45
Potassium (mg)	160	161	167
Calcium (mg)	122	124	129
Magnesium (mg)	11	11	11
Phosphorus (mg)	96	97	99
Iron (mg)	0.03	0.02	0.03
Zinc (mg)	0.4	0.4	0.5
Selenium (µg)	1	1	1

(The Dairy Council-The Nutritional Composition of Dairy Products (http://www.milk.co.uk/pdfs/NutritionalCompositionDairy.pdf)) (Accessed 21 March 2007)

1.3 Daily intake of milk

Data detailing the daily intake of milk by men and women of different age groups in the UK is given in Table 3.

Table 3 Daily intake of milk (g) (whole, semi-skimmed and skimmed) by men, women and all respondents aged 19-49 years old

Age	19-24	25-34	35-49
Men	133	220	239
Women	153	162	208
All	143	192	222

(Hoare *et al*., 2004)

1.4 Meeting nutrient requirements of humans with milk

Data detailing the contribution of milk and milk products to the mean daily intake of nutrients is given in Table 4.

Table 4 Contribution of milk and milk products to mean daily intake of nutrients

Nutrient	Contribution (%)	Source
Vitamin B_{12}	36	Milk & milk products (18% from semi-skimmed milk)
Riboflavin	33	Milk & milk products (16% from semi-skimmed milk)
Phosphorus	24	Milk & milk products (9% from semi-skimmed milk)
Calcium	6	Whole milk
	17	Semi-skimmed milk
	4	Skimmed milk
	11	Cheese
	3	Yoghurt
Zinc	17	Milk and milk products (6% from semi-skimmed milk)
Vitamin A	14	Milk & milk products
Potassium	13	Milk & milk products (6% from semi-skimmed milk)
Magnesium	11	Milk & milk products (5% from semi-skimmed milk)
Sodium	8	Milk & milk products
Iron	1	Milk & milk products

(Henderson *et al*., 2003)

2 Effect of nutrients in milk on bone health

Human nutrition plays a major role in bone development and bone strength, with calcium, vitamin D and proteins being particularly important (Bonjour, 2005). The presence of these nutrients in the human diet is also vital to aid in the prevention of osteoporosis.

In a comprehensive review by Cashman (2006) examining the importance of minerals in milk (calcium, phosphorus, magnesium, sodium, potassium and zinc) in human bone health, it was concluded that there is good evidence that a number of minerals present in milk have an important role in bone metabolism and bone mass. However, it was also concluded that the evidence base linking benefits to bone from an increased intake of minerals in the diet is mainly based on data from human studies that have used various forms of the mineral, not just milk-based forms of mineral.

2.1 Vitamin D

In a comprehensive report to examine the relationship between bone health and osteoporosis (United States Department of Health and Human Services, 2004), the role of vitamin D in facilitating the absorption and utilisation of calcium was examined. Vitamin D is mainly produced in humans by exposure of skin to sunlight, resulting in the alteration of precursors to active vitamin D, 1,25 dihydroxy vitamin D. This is the principal hormone responsible for regulating calcium absorption from the small intestine and maintenance of plasma calcium levels via bone resorption and formation. An individual's level of exposure to adequate sunlight and degree of skin pigmentation are major factors in determining the ability to synthesise adequate amounts of vitamin D (Phillips, 2004). A deficiency of vitamin D in the human diet can cause diseases such as rickets in children, osteomalacia in adults and contribute to osteoporosis (Phillips, 2004).

In a review undertaken in the United States, the synergistic relationship between calcium and vitamin D was examined (Heaney, 2007) and it was highlighted that approximately 85% of the female population failed to receive the recommended calcium intake after childhood, and between 65-100% of the population were deficient in vitamin D after mid-life.

2.2 Calcium

Calcium is an essential mineral for bone health and its roles in human health were reviewed by Cashman (2002a). The majority of calcium in the body (>99%) is found in bone and teeth, therefore any deficiency in calcium levels has a direct impact on bone health, resulting in reduced bone mass and disease, such as osteoporosis. Results from UK surveys which examined dietary sources of calcium for the human diet indicate that the largest proportion comes from milk and milk products (>40% in adults) (Food Standards Agency, 2002; Theobald, 2005). The bioavailability of calcium is greater from milk and milk products than from any other foodstuff (Heaney, 1998). In a review of the evidence relating dairy foods to bone health in the U.S. population (Weinsier and Krumdieck, 2000), it was concluded that the nutrients present in dairy foods which have a direct influence on calcium excretion and skeletal mass can vary substantially. These authors also reported on other studies which suggested that milk appears to be more beneficial in terms of bone health than other dairy foods.

Whilst examining the causes of poor bone health in some children, an extended absence of milk from the children's diet was reported and Black *et al*. (2002) related this to their small stature and poor bone health. Studies by Cashman and Flynn (1999) and Flynn (2003) to examine the effect of an increased calcium intake in adults above the normal dietary intake level have indicated an enhanced development and maintenance of bone, with a possible reduction in the occurrence of osteoporosis in later life.

The average daily intake of calcium by the UK population is 888 mg/day (Theobald, 2005), with the main dietary source being milk and milk products (43% of calcium intake). However, dietary intake of calcium in some population groups is below average, namely amongst male and female 19-24 year olds, with 8% of women consuming less than 400 mg/day (Theobald, 2005).

2.3 Phosphorus

In the human body, approximately 85% of phosphorus (P) is bound in the skeleton (Ilich and Kerstetter, 2000) with some studies indicating that insufficient levels of dietary P can have an adverse effect on bone health (Heaney, 2004). Phosphorus is an essential nutrient for humans but concerns that excessive amounts of P may be detrimental to bone have been raised by Ilich and Kerstetter (2000). This effect may be attributed to the fact that an increase in dietary P results in a concomitant increase in serum P concentration, which can result in a reduction in serum ionized calcium and an elevated secretion of parathryroid hormone, which can potentially result in bone resorption. Levels of P have increased in recent years due to the addition of phosphate salts to foods, in particular, their inclusion in soft drinks (Whiting *et al.,* 2001). It is this increase in dietary P intake through food that has resulted in concern that excessive amounts of the mineral may be detrimental to bone health (Cashman, 2006). Conversely, in an earlier review by Calvo and Park (1996) examining the changing P content of the U.S. diet and its potential for adverse effects on bone in humans, it was concluded that there were no clinical studies linking a high P consumption in the human diet (with or without a sufficient intake of calcium), to increased rates of bone loss in humans. However, the use of animal models has strengthened the theory that excess phosphate is detrimental to bone health (Katsumata *et al*., 2005; Huttunen *et al*., 2006).

2.4 Magnesium

Magnesium is essential for the normal function of the parathyroid gland and for vitamin D metabolism, while calcium homeostasis is controlled in part by a magnesium-requiring mechanism which releases parathyroid hormone. Up to 60% of the body's level of magnesium is contained within the bone, where it contributes to bone quality and the improvement of bone density. A deficiency of magnesium can be a possible factor in the development of bone diseases such as osteoporosis (Rude, 1998). In a comprehensive review by Cashman (2006), it was noted that relatively few trials have examined the effect of magnesium supplementation on bone mass and metabolism in humans and this is an area requiring further research.

2.5 Zinc

Zinc is an essential component of over two hundred metallo-enyzymes in the body and hence is involved in nucleic acid synthesis, protein digestion and synthesis, carbohydrate metabolism and bone metabolism. It plays a role in stabilising the structure of organic components and membranes and helps optimise the body's defence system. A survey on the mineral intake by Irish adults aged 18-64 was conducted between 1997-1999 (Hannon *et al.*, 2001). Data indicated that mean daily intakes of zinc were significantly higher for men than for women (11.6 mg/day and 8.5 mg/day respectively) (Table 5). Differences in the intake of zinc for men and women were also significant ($P < 0.001$) for all age groups.

Table 5 Mean daily intake of zinc (mg) from all sources by age and sex

	Men				Women			
Age (yrs)	**18-35**	**36-50**	**51-64**	**All**	**18-35**	**36-50**	**51-64**	**All**
Mean	11.1	12.0	11.6	11.6	7.8	8.9	9.0	8.5
s.d.	4.3	4.7	4.1	4.4	4.2	5.9	4.2	5.0
Median	10.2	11.4	10.5	10.8	6.9	7.9	8.0	7.5

(Hannon *et al*., 2001)

A study on the trace elements in bovine milk by Oberleas and Prasad (1969) reported levels of zinc in bovine milk ranging from 300-400 µg/100 ml to 500-600 µg/100 ml.

Zinc plays an important role in enzyme production and in particular for the enzymes responsible for bone metabolism (Cashman, 2006). The enzymes that play a role in bone metabolism in rabbits have been reported to require zinc in order to function correctly (Swann *et al*., 1981). Approximately 30% of zinc in humans is stored in bone (Moser-Veillon 1995) and it is incorporated into alkaline phosphatase and collagenase, which are used in bone calcification and bone resorption and remodelling respectively (Swann *et al*., 1981).

2.6 Sodium

Sodium, together with potassium, is an essential mineral for regulating body fluid balance. Increasing levels of body sodium, by increasing the consumption of salt (sodium chloride) in the diet, can result in an increased calcium excretion in urine (Cashman and Flynn, 2003). Although the relationship between sodium intake and calcium urinary excretion has been established, studies on its effect on bone health have only been conducted over short periods of time and more extensive clinical evidence is required before conclusions can be drawn (Cashman and Flynn, 2003; Ilich and Kerstetter, 2000).

2.7 Potassium

Potassium has been demonstrated to have a positive effect on bone health, with inclusion of potassium salts in the diet resulting in a reduction in urinary calcium excretion (Morris *et al*., 1999). Sellmeyer *et al.* (2002) examined the impact of offering increased levels of sodium chloride in the diet on urine calcium excretion with postmenopausal women offered 1) high salt diet plus a placebo compared with those offered 2) high salt diet plus 90 mmol/day potassium citrate. Data demonstrated that with women offered a high salt diet plus a placebo, urine calcium excretion increased by 42 ± 12 mg/day (mean ± s.e.m.) and decreased 8 ± 14 mg/day with women offered the high salt diet plus potassium citrate ($P = 0.008$).

2.8 Protein

In a review by Swiss researchers on the effect of dietary protein on bone health (Bonjour, 2005), it was stated that a deficiency of dietary protein can cause a marked deterioration in bone mass, microarchitecture and strength, which subsequently leads to osteoporosis. Conversely, other researchers have claimed that the intake of proteins in the human diet via animal sources can be a risk factor in terms of osteoporosis, due to the production of renal acid which would cause the bone mineral to dissolve (Barzel, 1995; Barzel and Massey, 1998). This claim was challenged by Bonjour (2005).

3 Effect of nutrients in milk on mood in humans

The World Health Organisation has identified psychiatric illnesses, for example anxiety and depression, as a major problem for the 21st Century. In a paper by researchers at the University of Wales Swansea (Benton and Donohoe, 1999), the effect of nutrients on human mood was examined. The main conclusions in this paper were that a) intake of carbohydrate is associated with an improvement in human mood b) poor mood leads to the eating of comfort foods, such as chocolate and c) a poorer mood can result from a deficiency of many micro-nutrients, especially thiamin and iron. According to these authors, the most quoted hypothesis regarding carbohydrate and depression is that of Wurtman and Wurtman (1989), who postulate that carbohydrate increases the rate at which tryptophan enters the brain, with a concomitant increase in levels of the neurotransmitter serotonin, which is responsible for regulating mood.

3.1 Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring hormone that is produced in the pineal gland of the human brain. Daylight induces a considerably lower plasma melatonin level, while at night the pineal gland increases its melatonin secretion (Brzezinski, 1997). The biochemical pathways by which melatonin exhibits its effects are comprehensively described by Beyer *et al*. (1998).

The mean concentration of melatonin in human serum varies with age and data presented in Table 6 clearly demonstrates this.

Table 6 Peak nocturnal melatonin concentration in human serum (pg/ml)

Age	<3 months	1-3 years	Young adults
Serum melatonin (pg/ml)	Very little	325	60

(Brzezinski, 1997)

In a review by Brzezinski (1997) the possible role of melatonin in relation to mood disorders, such as seasonal affective disorder (SAD) was addressed. However, the mechanism by which this occurs is unknown, with clinical evidence from comparative studies examining the pattern of melatonin secretion and studies examining phototherapy for mood disorders being the only indicators linking melatonin to mood in humans.

In a study by American researchers (Jean-Louis *et al*., 1998), the effects of melatonin on sleep, mood, and cognition in elderly subjects who reported sleep-wake disturbances was examined. A total of 6 mg melatonin was administered 2 hours before bedtime, over a ten-day trial, which was reported to improve sleep quality. The total sleep time was not significantly increased and wake within sleep was not significantly reduced with melatonin supplementation. A reduction in the incidence of depressed moods was also reported with melatonin supplementation. The authors concluded that melatonin can improve some aspects of sleep, memory and mood in the elderly with short-term use.

Humbert and Pevet (1994) reported that the quality of sleep in humans is dependant upon a naturally oscillating melatonin secretion pattern and a rapid increase in the concentration of melatonin is responsible for the onset of sleep in humans (Beyer *et al*., 1998). Brzezinski (1997) also reported a link between melatonin and sleep in humans, with evidence for this hypothesis coming from controlled clinical trials, as reviewed by these authors (Table 7).

In a more recent review by Indian researchers (Malhotra *et al*., 2004) which examined the therapeutic potential of melatonin, a number of studies examined the relationship between melatonin and sleep disturbances. They concluded that data from clinical trials have demonstrated that melatonin is effective in treating sleep disorders related to the circardian rhythm.

Finnish researchers examined the effect of offering melatonin-rich night-time milk on sleep and activity in elderly people, assuming that their own ability to produce melatonin was reduced due to old age and decreased health (Valtonen *et al*., 2005). The night-time milk contained 10-40 ng/l melatonin and was consumed as a drink to accompany meals at 0.5 l/day, being equivalent to an intake of 5-20 ng melatonin daily. The effect of offering night-time milk was compared with normal milk (control) for 8 weeks. In the first study, which was conducted in spring time, it was concluded that offering night-time milk v normal milk had no effect on sleep quality. The fact that the study was conducted in the spring time when patients had more exposure to light masked the effects of offering night-time milk. Therefore a second study was conducted between October and February, when the nights were longer and patients had a reduced exposure to light. Patients involved in this study reported a significant increase in both morning and evening activity upon the consumption of night-time milk. In conclusion, it was stated that even very low doses of melatonin may benefit some elderly people due to an increased day time activity, possibly a result of an improved sleep quality. Valtonen *et al*. (2005) mentioned how further studies were required in this area. Countries marketing night time milk include the Republic of Ireland, UK, Finland and Japan.

Table 7 The effect of exogenous melatonin on sleep variables and sleep disturbances

Study	Year	Subjects	Melatonin administration		Effect
			Dose & route	Timing & duration	
Cramer *et al*.	1974	15 normal	50mg intravenously	9.30 pm	Decreased sleep onset latency
Vollrath *et al*.	1981	10 normal	1.7 mg intravenously	Daytime	Induction of sleep
Lieberman *et al*.	1984	14 normal	3X80 mg over 2 hour period	Daytime	Reduced alertness, increased fatigue and sleepiness
Dahlitz *et al*.	1991	8 with delayed sleep phase syndrome	5 mg orally	10 pm for 4 weeks	Earlier onset of sleep and wake up time
Haimov *et al*.	1995	26 elderly insomniacs	2 mg orally sustained v fast release	2 hours before bed time for 1 week	Increased efficiency and duration of sleep in sustained release group. Improved initiation of sleep in fast-release group
Garfinkel *et al*.	1995	12 elderly insomniacs	2 mg orally controlled release	At night for 3 weeks	Increased efficiency of sleep. No effect on total sleep time
Oldani *et al*.	1994	6 with delayed sleep phase syndrome	5 mg orally	For 1 month	Advanced onset of sleep
Dollins *et al*.	1994	20 young	0.1 or 0.3 mg orally	Midday	Increased duration of sleep , decreased sleep-onset latency
Zhdanova *et al*.	1995	6 young	0.3 or 1.0 mg orally	6 pm, 8 pm or 9 pm	Decreased sleep-onset latency
Wurtman and Zhdanova	1995	9 elderly with insomnia	0.3 mg orally	30 minutes before bedtime	Increased efficiency of sleep, decreased sleep-onset latency

(From Brzezinski, 1997)

A report by the Agency for Healthcare Research and Quality in America (Buscemi *et al*., 2004), to examine the effect of melatonin for the treatment of sleep disorders, mainly using evidence from controlled clinical trials concluded the following:

1. Evidence suggests that short-term use of melatonin is not effective in treating the majority of primary and secondary sleep disorders. However, there is some evidence to suggest that short-term use of melatonin is effective in treating delayed sleep phase syndrome.

2. There is no evidence to suggest that melatonin is effective in addressing sleep disturbance in relation to jet lag and tiredness due to shift work.

3. The evidence suggests that the short-term use of melatonin is safe.

4. Evidence suggests that exogenous melatonin has a short half-life.

5. There is a link between exogenous melatonin and the sleep cycle in humans.

3.2 Vitamin E

In the body, vitamin E has a primary role as an antioxidant but it also interacts with the prostaglandin synthetase enzyme complex to reduce inflammation and has a role in DNA synthesis. There are limited studies on the effect of vitamin E on mood. Belgian researchers examined serum vitamin E levels in major depressed patients and normal volunteers by assessing serum vitamin E concentrations in 26 healthy volunteers and 42 major depressed patients (Maes *et al*., 2000). Data demonstrated that patients with major depression had significantly lower serum vitamin E concentrations than control groups.

3.3 Selenium

Selenium (Se) is essential for human health in that it is incorporated as a component of key metabolic pathways such as thyroid hormone metabolism, antioxidant defence system and immune function. Blood serum levels of Se have declined in recent years in both the UK and Europe, which could have implications for human health (Brown and Arthur, 2001). The reduced Se intake in both the UK and other European countries may in part be attributed to the decreased importation of Se rich wheat from North America (Brown and Arthur, 2001).

Dietary intake of Se in the UK has declined from 60 µg/day in 1976 to 34 µg/day in 1997 (Rayman, 2000). The UK reference nutrient intake (RNI) for selenium is 70 µg/day for men and 60 µg/day for women, so it is recommended that Se levels should be increased through the intake of foods such as milk and milk products (Cottrill and Givens, 2003) The rate of decline in UK Se levels has been described by Jackson *et al.* (2003) and details are included in Table 8.

Table 8 Estimated Selenium intakes in the UK

Year	Intake (µg/d)
1974	60
1985	63
1991	60
1994	43
1995	29-39
1997	39

(Jackson *et al.*, 2003)

Daily consumption of milk in many countries has decreased, with levels in the UK falling from 2733 ml/person/week in 1975 to 1802 ml/person/week in 2000 (Buttriss, 2002). Table 9 outlines blood serum Se concentrations in a number of European populations as described by Brown and Arthur (2001).

Table 9 Serum Se concentrations of several European populations

Country	**Serum Se concentration µmol/l (s.d.)**
UK	1.01 (0.16)
Greece	0.79 (0.17)
West Germany	0.81 (0.12)
Sweden	1.02 (0.13)
Spain	1.10 (0.17)
Denmark	0.98 (0.18)
France	1.00 (0.18)
Belgium	1.07 (0.16)
Netherlands	1.07 (0.18)

(Brown and Arthur, 2001)

Murphy *et al*. (2002) conducted a trial to examine the Se intake of Irish adults (aged 18-64 years) with 662 men and 717 women and concluded that there was a significant prevalence of inadequate Se intake in Irish adults. These authors also examined the contribution of different food groups to Se intake in Irish adults and reported a contribution of 30% from meat and meat products, 24% from bread and rolls, 11% from fish and fish products and 9% from milk and yoghurt. In an effort to assess whether there was a sub-clinical deficiency of Se in the British population, Benton and Cook (1990) conducted a study with 50 subjects, who received either a placebo or 100 µg Se daily for 5 weeks. The authors concluded that the people who received Se supplementation had a substantial improvement in mood (Profile of Moods States questionnaire) after both 2.5 and 5 weeks, compared with the control group offered the placebo.

The British Nutrition Foundation (2001) conference examined the relationship between food and mood, and referred to a number of studies that demonstrated the beneficial effect of Se on mood, at least when the Se status was marginal. For example, Finley and Penland (1998); Hawkes and Hornbostel (1996) and Benton and Cook (1991) all reported an association between low Se status and an increased incidence of depression and other poor mood states. Benton (2002) reported mood as being the clearest example of an aspect of psychological functioning that is modified by Se intake, although the process by which this occurs is unclear.

In a more recent study, involving a much larger number of human subjects, 501 UK participants aged between 60-74 years were randomly allocated to either 100, 200 or 300 µg Se/day in the form of a high Se yeast or a placebo yeast (Rayman *et al*., 2006). The mood of the test subjects (Profile of Moods

States-Bipolar Form questionnaire), quality of life (questionnaire) and plasma Se were measured at baseline and after six months. Data demonstrated that Se supplementation resulted in a significant increase in plasma Se above that measured at baseline ($P < 0.001$). However, there was no significant difference in mood scores with Se supplementation, and a similar finding was reported when quality of life was assessed. The authors of this study concluded that there was no evidence to suggest that Se supplementation enhanced mood or quality of life in the elderly volunteers. Although the conclusions from this study were contrary to previous findings by other researchers, the authors were confident of their conclusions in view of the much larger sample size and longer period of Se supplementation than other studies reported in the literature.

3.4 Zinc

There are limited data available in the scientific literature on the direct relationship between zinc in the diet and human mood. A number of reports have demonstrated that zinc plays an antidepressant role with rodents (Levenson, 2006) and it has been reported that findings from human trials were consistent with those of animal trials (Nowak *et al*., 2003). In the study by Nowak *et al*. (2003), data obtained demonstrated that supplementing the diet with zinc augmented antidepressant drug therapy by more than 50%.

In a review of the role of zinc as an antidepressant in humans (Levenson, 2006), it was reported that low levels of zinc in human serum were linked to major depression and that treatment with zinc had positive implications for humans suffering from depression. Researchers are now examining the mechanism whereby zinc assists in the uptake of serotonin in the brain. Levenson (2006) reported on a number of studies that linked depression with low serum zinc levels (Maes *et al*., 1994; Maes *et al*., 1997; Maes *et al*., 1999; Nowak *et al*., 1999). In these studies, it was found that patients suffering from major depression had serum zinc levels 12-16% lower than controls and that the severity of the symptoms of depression was also linked with zinc levels. In a later paper, Nowak *et al*. (2005) conducted a preliminary clinical study to examine the effect of zinc supplementation on antidepressant therapy. Data indicated the important role of zinc homeostasis in the therapy of depression and also indicated potential clinical antidepressant activity. However, in the review by Levenson (2006), it was concluded that the exact role of zinc in the regulation of human mood is not yet fully understood.

The North/South Ireland Food Consumption Survey (Hannon *et al*., 2001) examined the mineral intake and the contribution of different food groups to mineral intakes of Irish adults aged 18-64 years old. In this survey, it was reported that milk and milk products contributed 14% of the mean daily intake of zinc and that a high proportion of women who participated in this study had intakes of zinc that fell below the average requirement.

3.5 *n*-3 Polyunsaturated fatty acids (PUFA)

The fatty acid α-linolenic acid C18:3 is an omega-3, or *n*-3 fatty acid that has to be taken into the human body via dietary means because it is not synthesized by humans, and is therefore considered essential in the diet. A deficiency of α-linolenic acid in the diet can result in symptoms including scaly and hemorrhagic dermatitis, hemorrhagic folliculitis of the scalp, impaired wound healing and growth retardation (Dietary Guidelines Advisory Committee Report, 2005). Alpha-linolenic acid is primarily obtained from plant sources including soybean oil, canola oil, walnuts, and flaxseed.

The *n*-3 PUFA have positive implications for human health and have been described as the 'beneficial' fatty acids in the past (Chow, 1992; Sargent, 1996). Research from a review by Williams (2000) suggests that a deficiency of *n*-3 PUFA may be linked with hypertension, inflammatory disorders, immune disorders, depression and neurological dysfunction. Puri *et al*. (2001) also demonstrated that the *n*-3 PUFA found in fish oil, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were therapeutic for depression.

3.6 Lactalbumin

Bovine milk proteins may be divided into caseins and whey or serum proteins, although other protein fractions are also present at very low levels. The casein and serum proteins can be separated by acidifying raw milk to pH 4.6 at 20°C, causing the caseins to precipitate. The serum proteins are found in the residual whey after the caseins are removed. The two main serum proteins are β-lactoglobulin (80%) and α-lactalbumin (20%). Alpha-lactalbumin has been identified as the specifier protein of the lactose synthetase system. Although it has no catalytic function, α-lactalbumin acts as the specific carrier protein in facilitating the action of galactosyl transferase. This enzyme combines with manganese glucose and UDP-galactose to form a dimer which accepts small concentrations of glucose in the formation of lactose under physiological conditions. Alpha-lactalbumin is present in cow's milk at a concentration of 1–1.5 g/l and has a globular structure in aqueous solution. Its amino acid composition is relatively rich in tryptophan (four residues per mole) and also in sulphur as sulphydryl groups in cystine and methionine amino acids. Unlike β-lactoglobulin, it contains no disulphide groups (no cysteine).

There is considerable evidence that serotonin (5-hydroxytryptamine or 5-HT) plays an important role in stress-related disorders such as depression. Studies have shown that depletion of L-tryptophan (Trp), the precursor of 5-HT induces symptoms of depression in vulnerable subjects (though not in healthy subjects) while administration of Trp improved mood, reduced aggression and had some therapeutic effect in mild and moderate depression (Merens *et al*., 2005). Several studies have attempted to increase 5-HT concentrations through dietary intervention by providing carbohydrate-rich/protein-poor diets to increase the ratio of Trp:large neutral amino acids (LNAA). However results have not been consistent (see reviews by Dye *et al*., 2000 and Benton and Nabb, 2003) and such diets providing high calorific intakes are not healthy if consumed on a daily basis.

A different dietary approach to increase Trp availability has been investigated by providing a diet containing high levels of α-lactalbumin (Markus *et al.,* 2002). Alpha-lactalbumin has the highest Trp concentration of all protein fractions and was found to almost double the Trp:LNAA compared to carbohydrate-rich/protein-poor diets. An α-lactalbumin enriched diet was found to improve mood and information processing and attenuated stress-induced cortisol-responses in stress-vulnerable subjects (Markus *et al.,* 2002). These responses could not be reproduced by Booij *et al.* (2006). They increased total Trp levels by the provision of α-lactalbumin in enriched milk-shake type drink but reported no or minimal effect on mood, although abstract visual memory and impaired simple motor performance were improved, independent of depression.

Brain serotonin is also involved in the regulation of sleep and cognitive processes, with sleep abnormalities and subsequent behavioural decline attributed in part to deficient brain serotonin activity (Markus *et al.,* 2005). Provision of α-lactalbumin-rich milk shake drinks with an evening meal and for supper were found to substantially increase the Trp:LNAA and this was associated with modest reductions in sleepiness and improved brain-sustained attention processes the following morning (Markus *et al.,* 2005).

3.7 Folate/Vitamin B_{12}

Folate co-enzymes within the body are involved in one-carbon transfer reactions including those involved in phases of amino acid metabolism, purine and pyrimidine synthesis and the formation of the primary methylating agent, S-adenosylmethionine. Folate, vitamin B_6 and vitamin B_{12} metabolism are linked via the enzyme methionine synthase (which requires vitamin B_{12} as a co-factor). Many researchers have reported a link between folate or vitamin B_{12} deficiency and depression, dementia (Hunter *et al.*, 1967; Botez *et al*., 1977; Carney and Sheffield, 1978; Abou-Saleh and Coppen, 1986; Hector and Burton, 1988; Sommer and Wolkowitz, 1988; Shorvon *et al*., 1980). For example, in the study by Botez *et al*. (1977), a group of 49 patients suffering from depression and with low levels of folate were selected. The patients were offered folate for seven to eleven months and results demonstrated that they did not suffer from fatigue as much as they did prior to supplementation and they were less easily distracted.

3.8 Thiamin

Like many of the other B vitamins in the body, thiamin, (in the form of the pyrophosphate ester) is a co-enzyme in a number of enzyme systems, especially those concerned with the metabolism of fat, protein and carbohydrate. In a study to examine the effect of thiamine supplementation on the health and general well-being of an elderly Irish population with a marginal thiamin deficiency, Smidt *et al*. (1991) concluded that taking 10 mg thiamin for six weeks led to a greater feeling of well-being and a reduction in the incidence of fatigue. Similar reports were given by UK researchers who examined thiamin deficiency in young females (Benton *et al*., 1997).

3.9 Iron

The majority of functional iron in the body is present in haem proteins (e.g. haemoglobin) which are involved with oxygen transport or mitichrondrial electron transfer. Iron may be stored, mainly in the liver, as iron storage proteins, ferritin and haemosiderin. Small amounts of ferritin are also present in the serum. It has been reported that 52% of females and 11% of males in the UK population are deficient in iron (serum ferritin levels below the accepted level of 20 ng/l) (Fordy and Benton, 1994). Benton and Donoghue (1999) reported that iron deficiency anaemia is associated with poor mood, lethargy and reduced attention focus.

4 Effect of nutrients in milk on immune health in humans

4.1 Selenium

Selenium is a constituent of selenoproteins and hence has important structural and enzymic roles within the body (Rayman, 2000). In addition, Se is essential for the proper functioning of the immune system and is found in significant amounts in immune tissues such as liver, spleen and lymph nodes (Rayman, 2000). A number of human diseases and conditions have been linked with Se deficiency including immunodeficiency (Reilly, 1998). Selenium is also important for the immune system of people who are suffering from different diseases or conditions. For example, research by Arthur (2002) into the effect of Se supplementation of the human diet on immune health, established an increased defence response to the polio virus by human lymphocytes. The subjects were administered with a live poliomyelitis vaccine 6 weeks from the start of the study and results demonstrated that those receiving the Se supplement had a greater recovery from the polio virus, with an enhanced production of cytokines and T-cells, than the control group offered a placebo (Arthur, 2002).

Furthermore, recent research has focused on the role of Se for immune health of individuals with HIV (human immunodeficiency virus), with Se being viewed as an essential nutrient for HIV patients (Rayman, 2000). A recent study by American researchers who evaluated the effect of Se supplementation on HIV positive patients demonstrated an increased T-cell count for Se supplemented groups compared to those offered a placebo (Hurwitz *et al*., 2007).

4.2 Zinc

Zinc plays a major role in the human immune system, being crucial in the normal development and function of cells involved in immunity, namely neutrophils and normal killer cells (Shankar and Prasad, 1998). Berger (2002) outlined the importance of zinc in promoting cell growth, antioxidant capacity and suppression of apoptosis, programmed cell death. Walsh *et al*. (1994) also reported on studies using animal models which demonstrated the requirement for zinc to maintain the integrity of the immune system. Walsh *et al*. (1994) also cited a number of references which demonstrated the drastic effect of suboptimal zinc levels for 30 days in the young adult mouse, namely a reduction in weight loss ranging from 20-25% and a 50-75% reduction in lymphocytes and macrophages in the spleen.

4.3 *n*-3 Polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA)

Essential fatty acids are required for the formation of healthy cell membranes, for proper development and functioning of the brain and nervous systems, and for the production of hormone-like substances called eicosanoids. Eicosanoids regulate many body functions such as blood pressure, blood viscosity, vasoconstriction, immune and inflammatory responses (Woods *et al*., 2005). In the world review of nutrition and dietetics as edited by Simopoulos (2003), the scientific evidence available regarding the importance of *n*-6:*n*-3 in the prevention and management of different diseases and conditions was considered. It was stated that increased quantities of *n*-3 fatty acids in the human diet exert suppressive effects on inflammatory and autoimmune diseases. Docosahexaenoic acid and EPA have been demonstrated to have therapeutic effects for autoimmune disease (Harbige and Fisher, 2001) and inflammatory effects (Grimm *et al*., 2002). Research over the past ten years has shown that CLA can influence immune function in animals (Roche *et al*., 2001), however there have been relatively few studies reported on its effect in humans. Nugent *et al*. (2005) assessed the effects of CLA on immune function in humans by supplementing the diet of healthy volunteers with two isomeric blends of CLA (50:50 *cis*-9, *trans*-11/*trans*-12, *cis*-10 blend and 80:20 *cis*-9, *trans*-11/*trans*-12, *cis*-10 blend). The improvements in immune function observed could not be solely attributed to CLA as similar results were observed with linoleic acid and the authors concluded that CLA supplementation had a minimal effect on the markers of human immune function but recommended that further research to elucidate the effects of individual isomers was needed.

4.4 Bioactive whey proteins and peptides - Micronutrients in bovine colostrum-lactoferrin, lysozyme, lactoperoxidase, peptides

In recent years the interest in the promotion of food products with health benefits has led to several studies into the potential beneficial effects of milk components, in particular the whey proteins and peptides. Beaulieu *et al*. (2006) reviewed the impact of these components on human health. These components and a description of their properties are identified in Table 10.

Table 10 Bioactive whey proteins in cow's milk

Protein	Concentration in cow's milk (g/l)	Bioactivity observed
β-lactoglobulin	2.0-4.0	ACE-I activity, opioid agonist, anti-microbial
α-lactalbumin	1.0-1.5	ACE-I activity, immunomodulation, anti-microbial, opioid agonist
Lactoferrin	<0.1	Anti-microbial, anti-inflammatory, anti-tumour, anti-angiogensis, bone cell proliferation, anti-oxidant, anti-thrombotic, opioid antagonist
Immunoglobulins	0.4-1.0	Increases glutathione, anti-microbial
Lactoperoxidase	0.03	Anti-microbial
Lysozyme	0.0004	Anti-microbial, synergistic effect with immunoglobulins and lactoferrin
Bovine serum albumin	0.1-0.4	Increases glutathione, stimulatory effect on splenocytes

(Compiled from Shah, 2000; Rowan *et al*., 2005; Beaulieu *et al*., 2006)

Minor components of bovine whey proteins lactoferrin, lyszoyme, the lactoperoxidase and immunoglobulins have been of recent interest because of their anti-microbial properties. Lactoferrin is the most studied of the whey proteins and is thought to be the best immunomodulator. It is an iron binding glycoprotein found in milk and other exocrine secretions of mammals such as the tears and saliva, and is also a constituent of the colostrum (Beaulieu *et al*., 2006). Bovine lactoferrin is homologous to human lactoferrin. The concentration of lactoferrin in bovine colostrum and milk is approximately 1.5-5 g/l and 0.1 g/l respectively, while in human milk and colostrum the reported levels are 2-4 g/l and 6-8 g/l, respectively, reflecting its importance to the newborn human infant (cited in Shah, 2000). Numerous studies to examine the role of lactoferrin in immunity have been conducted and the major observations from these have been reviewed by Van Hooijdonk *et al*. (2000), Wakabayashi *et al*. (2006) and Beaulieu *et al.* (2006). These roles are diverse and dependent upon the conditions for which lactoferrin is used. The capacity of lactoferrin to act both as an immunosuppressive and an immunostimulatory agent are among its most important activities. Lactoferrin has been shown to help modulate immunity by acting as a regulatory nutrient to control cytokine production (Beaulieu *et al*., 2006). The increase of tumour-inhibiting cells following oral dosing of mice with lactoferrin has demonstrated a protective role of the protein in control of tumour metastases, while its ability to interact with gut-associated lymphocytes suggests that lactoferrin can act as an immunostimulatory factor on the mucosal immune system.

Lysozyme is an anti-microbial enzyme found in human milk at a concentration of 0.4 g/l but at a much lower concentration in bovine milk (Table 10). Because of the large concentration of lysozyme in the albumen of chicken eggs (1-3 g/l), most studies have been carried out using lysozyme from this source (Expoisito and Recio, 2006). Lysozyme acts by hydrolysing the β(1-4)-linkage between muramic acid and *N*-acetylglucosamine of mucopolysaccharides in bacterial cell walls (Fox and McSweeney, 1998). The anti-microbial activity of lysozyme is not solely because of its catalytic action but also because of a non-enzymic effect related to changes in its surface hydrophobicity (reviewed by Masschalck and Michiels, 2003). Combinations of lysozyme and lactoferrin are more bacteriostatic than either of the proteins alone (Suzuki *et al*., 1989) and several clinical studies (reviewed by Goldman (1989); Davidson (1996) and Pakkanen and Aalto (1997)) have demonstrated efficacy of immune milk preparations in the therapy of gastrointestinal diseases.

Lactoperoxidase is another enzyme with a major anti-microbial role in milk. The enzyme, in the presence of hydrogen peroxide (H_2O_2), catalyses the oxidation of thiocyanate (SCN^-) producing an intermediate product with anti-microbial properties (Shah, 2000). The concentration of the enzyme in bovine milk (Table 10) is much higher than in human milk although the level in infant salvia is high and probably contributes to the peroxidise activity in the human intestine (Van Hooijdonk *et al*., 2000). The lactoperoxidase enzyme is one of the indigenous antimicrobial agents present in bovine milk. Many *in vitro* studies have shown its bacteriostatic and bactericidal effects against a broad range of micro-organisms and other reported biological functions include anti-viral activity and tumouricidal activity (cited in Van Hooijdonk *et al*., 2000). The use of lactoperoxidase as a natural preservative in dairy systems has been commercially applied for a number of years (De Wit and Van Hooijdonk, 1996) and it has seen application in oral hygiene products such as toothpaste, mouthwashes and artificial salvia for the maintenance of non-cariogenic and non-infectious flora (Van Hooijdonk *et al*., 2000).

Immunoglobulins (Ig) are antibodies that are synthesised by mammals in response to antigenic or immunogenic stimuli (e.g. bacteria) and thus provide protection against infection (Mehra *et al*., 2006). Bovine colostrum is a rich source of Ig, about 100 times higher than the milk, where the main classes are IgG1, IgM, IgA and IgG2, Table 10 (Beaulieu *et al*., 2006). The principal Ig in bovine milk is IgG1 while in human milk it is IgA. The calf is born without Ig in its blood serum but a high concentration in the colostrum provides antibodies within 3 hours of suckling, increasing passive immunity. Human foetuses obtain Ig *in utero* but human colostrum also contains high levels (Fox and McSweeney, 1998).

The role of immunoglobulins from bovine milk in human health has been reviewed by Mehra *et al*. (2006). These authors examined evidence for the proposed health benefits of immunoglobulins in the preventative treatment of human infectious diseases. Studies indicated that immunoglobulins were effective in the treatment of orally medicated infections and that their inclusion in the diet could have positive implications for human health. Bovine colostrum could play a role in the treatment of common gut disorders such as Crohn's disease and irritable bowel syndrome (Playford *et al.*, 2000). *In vitro* and *in vivo* studies with rats indicated that supplementing the diet with colostrum could result in a reduction in injury to the small intestine.
Recent research has focused on the potential benefit of bovine colostrum on body composition and performance of athletes, with data suggesting that

colostrum supplementation may increase lean body mass in athletes, which would have positive implications for their performance (Antonio *et al*., 2001).

4.5 Melatonin

Brzezinski (1997) reported that melatonin promotes an enhanced immune response in humans, with evidence for this emanating from animal studies and a few uncontrolled studies in human subjects. In a recent review by Carrillo-Vico *et al*. (2005), the multiple actions of melatonin on the human immune system in relation to infection, inflammation and autoimmunity were examined. These authors also examined the relationship between melatonin, immunity and cancer. Carrillo-Vico *et al*. (2005) concluded that although the review of the literature indicates that the majority of melatonin effects appear to be beneficial in relation to the immune response in humans, it is only when a complete understanding of the synthesis and actions of melatonin in the immune system are understood, that benefits and potential side effects of melatonin will become apparent.

5 Effect of nutrients in milk on antioxidant activity in humans

Antioxidants are compounds that react with and neutralize the free radicals that could otherwise lead to cellular damage and off flavours, odours and appearances in food products. Free radicals are formed during the oxidation process when molecules are split to give products with unpaired electrons and they are highly reactive. Antioxidants can work by a number of pathways, for example by preventing the formation of radicals or by scavenging radicals or peroxides (oxidation intermediary products). Antioxidants present in milk include the enzymes superoxide dismutase and catalase, lactoferrin, and vitamins C and E in the form of tocopherols and tocotrienols (Lindmark Månsson and Åkesson, 2000). These authors concluded that a greater understanding of the mechanisms whereby the complex series of reactions of pro-and antioxidants occur would assist in identifying the importance of antioxidants in milk for human health.

5.1 Superoxide dismutase

The mechanism whereby superoxide dismutase acts as an antioxidant in milk is by catalysing the superoxide anion to hydrogen peroxide as follows:

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

Superoxide dismutase contains either copper/zinc, iron or manganese (Fridovich, 1986) with copper/zinc superoxide dismutase being present in bovine milk (Korycka-Dahl *et al*., 1979). Lindmark Månsson and Åkesson (2000) reported that the concentration of superoxide dismutase in bovine milk is probably not influenced by factors such as stage of lactation, age of cow or time of milking (morning or night) and its concentration in bovine milk is about 100 times lower than that of bovine blood (Hoolbrook and Hicks, 1978).

5.2 Catalase

Catalase works as an antioxidant by decomposing hydrogen peroxide as follows:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

The cream of milk contains the majority of catalase (60%), with skim milk having 40% catalase and it has been reported that the average catalase activity in raw milk was 1.95 IU/l (Lindmark Månsson and Åkesson, 2000).

5.3 Selenium

Selenium plays a vital role as an antioxidant in both human and animal health, constituting an essential component of the enzyme glutathione peroxidase, which protects cell membranes against oxidative damage by free radicals (Cashman, 2002b). Lindmark Månsson and Åkesson (2000) reported the presence of glutathione peroxidase in cow's milk at levels of 12-32 U/ml and that its activity is related to Se concentration. The activity of glutathione peroxidase and the Se content of cow's milk decreases with stage of lactation, reaching a plateau one month post-partum (Hojo, 1986). The glutathione peroxidase in cow's milk has a reported 27% peroxidase activity (Lindmark Månsson and Åkesson, 2000).

5.4 Vitamin E

Vitamin E is a fat soluble vitamin which acts as an antioxidant, protecting against the effects of lipid oxidation of cell membranes (Givens *et al*., 2003). It exists in several forms, with the most important being α-tocopherol, which is regarded as the form most commonly referred to as true vitamin E (Givens *et al*., 2000). Research has demonstrated a correlation between vitamin E and Se in their role as antioxidants in both animal and human health (Smith *et al*., 1997). Vitamin E can act to scavenge free radicals in the lipid phase of milk (Lindmark Månsson and Åkesson, 2000) as α-tocopherol is an important lipid-soluble antioxidant. Concentrations of α-tocopherol in bovine milk can range from 0.2-0.7 mg/l (Jensen, 1995) and levels are reduced substantially from the initial milk (colostrum) to four days post-partum (1.9 mg/l to 0.3 mg/l respectively) (Hidiroglou, 1989).

5.5 Vitamin C

Vitamin C in the form of ascorbic acid can act to scavenge free radicals in the aqueous phase of milk (Lindmark Månsson and Åkesson, 2000). Water soluble-vitamins are lost during the heating and storage of milk, whilst fat soluble vitamins are more stable (Korhonen and Korpela, 1994).

5.6 Zinc

Zinc has an antioxidant role in protecting cells from oxidative injury, in particular transition metal and protein oxidations (Powell, 2000). Zinc also plays a crucial role in acting as an antioxidant in maintaining skin health, by protecting the skin from UV radiation and assisting wound healing. Zinc is often incorporated into medical products for topical application to wounds by promoting healing through its involvement in antioxidant mechanisms (Rostan *et al*., 2002).

5.7 Melatonin

Melatonin also plays a role in terms of scavenging free radicals, acting as an antioxidant, as described by American researchers (Beyer *et al*., 1998). The role of endogenous and exogenous melatonin in neutralising free radicals has been examined by many researchers (e.g. Tan *et al*., 1994; Pieri *et al*., 1996; Pappolla *et al*., 1997). In a review by Brzezinski (1997), it was reported that in order for melatonin to act as a potent antioxidant in humans, concentrations required would by far exceed peak night-time concentrations in human serum and hence this effect may only be achieved at pharmacologic concentrations. Beyer *et al*. (1998) concluded that the precise method by which melatonin exhibits antioxidative effects is not fully understood. In a later review by Malhotra *et al*. (2004), it was reported that there is some evidence to suggest that melatonin can act as an antioxidant, with possible beneficial effects in terms of Alzheimer's disease, Parkinsons, cardiovascular, gastrointestinal and renal disorders.

6 Factors affecting levels of beneficial nutrients in milk

6.1 Vitamin D

6.1.1 Effect of season

Levels of vitamin D in cow's milk can vary with season (Kirchgessner *et al.*, 1967), with concentrations ranging from 0.06 IU/g fat (winter) to 0.23 IU/g fat (summer) reported in a study by Kurmann and Indyk (1994). The animal however is capable of synthesising enough vitamin D to attain adequate levels and therefore does not require dietary supplementation to meet the recommended requirements (Agnew and Newbold, 2002).

6.1.2 Effect of diet

Vitamin D may be present in relatively small amounts in herbage, with an increased content in mature herbage as opposed to younger material (McDonald *et al*., 1995). However, a significant amount of vitamin D (75%) is metabolised in the rumen of cattle offered high forage diets and offering a high concentrate diet would not result in any additional benefit (Weiss, 1998).

6.2 Calcium

6.2.1 Effect of season

Walker *et al*. (2006) examined the concentration of minerals in cow's milk throughout different seasons in Australia (April (mid autumn), July (mid winter), October (mid spring) and January (mid summer)). The minimum level of calcium was 864 mg/kg milk while the maximum level was 1310 mg/kg milk. The mean concentration of calcium for the four seasons examined was 1107, 1044, 1170 and 1110 mg/kg milk respectively, with concentrations being significantly different ($P < 0.05$) between seasons, with the exception of milk from cows in mid autumn and mid summer.

6.2.2 Effect of diet

Knowles *et al*. (2006) provided an overview of how minor milk components can be modified in grazing dairy cattle and concluded that dairy cows had a limited response to nutritional supplementation in studies designed to modify the calcium levels in milk on-farm. There are products available in New Zealand (CalciTrim™) that allows the fortification of milk calcium levels from ~1100 to 2050 mg Ca/litre. However, these products are produced by the addition of calcium to the milk during processing and it does not represent a natural means to increase milk calcium levels.

6.2.3 Effect of breed

The concentration of calcium in milk from Jersey cows is greater than Friesian cows (Davis *et al*., 2001).

6.3 Phosphorus

6.3.1 Effect of season

Walker *et al*. (2006) examined the concentration of P in cow's milk throughout (April (mid autumn), July (mid winter), October (mid spring) and January (mid summer)) in Australia. Minimum and maximum levels of P were 640 mg/kg milk and 1040 mg/kg milk respectively, with a mean of 885 mg/kg milk. The mean concentration of P for the four seasons examined was 859, 919, 975 and 896 mg/kg milk respectively, with concentrations being significantly higher ($P < 0.05$) in mid spring (October) compared to all other seasons.

6.3.2 Effect of diet

The main source of P on dairy farms in Northern Ireland is concentrate, with over 3025 tonne of P being imported onto farms in concentrate feeds, with almost half of that being removed in the milk (1539 tonne) (Foy *et al*., 2002). Although forages are a main constituent of the animal diet in the UK, the P content of forage is rarely reported or analysed. In a study to examine the P concentration of silages from 36 dairy farms in Northern Ireland sampled in 2003, P values ranged from 1.4 to 3.9 g P/kg dry matter (mean 3.1 g P/kg dry matter) (Personal communication, Dr Conrad Ferris, AFBI Hillsborough). Dairy cow rations generally contain high levels of P because it is required for milk production, with approximately 60% of P being excreted in faeces (Ferris *et al*., 2005). These authors reported on methods to improve the efficiency of nitrogen and P use in livestock systems via dietary modification. In a review of studies where different levels of dietary P were offered to dairy cows, Ferris *et al*. (2005) concluded that dietary P levels ranging between 3.5 and 4.2 g P/kg dry matter were sufficient for dairy cows. A summary of the main findings of this review are presented by Ferris *et al*. (2005).

Further research at the Agricultural Research Institute of Northern Ireland (now part of the Agri-Food and Biosciences Institute) was undertaken to examine the effect of managing high genetic merit Holstein-Friesian dairy cows on grass-based diets with "normal" or "reduced" levels of P in the diet (Ferris *et al*., 2005). "Normal" and "reduced" winter diets offered (70:30 grass silage and maize silage (dry matter basis) plus concentrate) contained 3.8 and 5.3 g P/kg dry matter respectively and summer concentrates were formulated to contain either 3.5 or 6.7 g P/kg dry matter. Data demonstrated that there was no significant effect of offering a “normal” or “reduced” P ration on P levels in milk (29.3 g/day and 29.7 g/day respectively). Ferris *et al*. (2005) recommended that dietary P levels can be safely reduced to 3.8 g/kg DM, without having a negative impact on animal performance or milk P content.

6.4 Magnesium

6.4.1 Effect of season

Walker *et al*. (2006) examined the concentration of magnesium in cows’ milk throughout different seasons (April (mid autumn), July (mid winter), October (mid spring) and January (mid summer)) in Australia. Minimum and maximum levels of magnesium were 73 mg/kg milk and 122 mg/kg milk respectively. The mean concentration of magnesium for the four seasons examined was 104, 97, 100 and 108 mg/kg milk respectively, with concentrations being significantly higher ($P < 0.05$) in January compared to July or October and significantly higher in April compared to July. The mean concentration of magnesium in milk was 98 mg/kg milk.

6.5 Zinc

6.5.1 Effect of season

The concentration of zinc in cow’s milk throughout the different seasons in Australia was examined by Walker *et al*. (2006). Minimum and maximum levels of zinc in milk were 2.15 mg/kg milk and 4.91 mg/kg milk respectively, with a mean of 3.46 mg/kg milk. The mean concentration of zinc in milk for the four seasons examined was 4.07, 2.73, 3.37 and 3.39 mg/kg milk for mid autumn, mid winter, mid spring and mid summer respectively. The concentration of zinc in the milk was significantly higher ($P < 0.05$) in mid autumn than all other seasons.

6.6 Potassium

6.6.1 Effect of season

Potassium levels in cow’s milk were higher ($P < 0.05$) in winter and spring than autumn or summer (1664, 1704, 1366 and 1575 mg/kg milk respectively, with an overall mean of 1534 mg/kg milk) (Walker *et al*., 2006).

6.7 Melatonin

6.7.1 Effect of daylight hours/season

Not all cows produce the same amount of melatonin. Night time milk is provided by herds that have been specifically selected for their ability to produce milk that is naturally higher in melatonin than regular milk. Eriksson *et al*. (1998) administered melatonin to dairy cows via the jugular vein as a bolus injection of 270 µg/kg and measured melatonin concentrations in both milk and serum at 2 and 5 minutes prior to administration and at 1, 2, 6, 10, 14, 30, 45, 75, 105, 165 and 225 minutes post-administration. The concentration of melatonin in milk increased rapidly after bolus administration and exceeded that in serum levels within 30 minutes. Of the four cows used for this study, the melatonin concentration in milk from the cow with the highest milk yield (28 l/day) had exceeded that in serum fifteen minutes after administration of the bolus, with a respective time of 45 minutes for the cow with the lowest milk yield (8 l/day). During the remaining times examined in the study, the milk melatonin levels exceeded those in serum. The authors also concluded that the duration of night-time melatonin rise was longer in winter than in spring, which indicates a seasonal effect. These authors also concluded that cows in late lactation may have a greater ability to accumulate melatonin in milk.

6.7.2 Effect of stage of lactation

Finnish researchers (Eriksson *et al*., 1998) compared the levels of melatonin in bovine milk and serum, using 4 Ayrshire cows housed indoors in a shed illuminated by lamps and offered silage and hay *ad libitum* at the beginning of the lactation. These authors reported that the nocturnal increase in milk melatonin was moderate ((mean ± sem) 7 ± 2 pg/ml at noon to 15 ± 1 pg/ml at night) and it did not correlate well with serum melatonin levels (7 ± 2 pg/ml at noon to 27 ± 7 pg/ml at night respectively). Conversely, six cows at a later stage in lactation were used in another study which demonstrated a clear and long-lasting nocturnal increase in melatonin level in both milk (12 ± 5 pg/ml at noon to 26 ± 7 pg/ml at night) and serum (9 ± 1 pg/ml at noon to 26 ± 3 pg/ml at night). This therefore suggests that stage of lactation may have an impact on serum and milk melatonin levels in dairy cows.

6.8 Vitamin E

6.8.1 Effect of diet

Flachowsky *et al*. (1997) reported increased levels of vitamin E in milk after cows were offered rations containing rapeseed, with similar findings being reported by Focant *et al*. (1998), when extruded rapeseed and linseed were offered to dairy cows. However, Focant *et al*. (1998) also reported a decreased resistance of milk to oxidation. Supplementing these dairy cow diets with 10,000 IU α-tocopherol daily increased milk α-tocopherol levels and increased the resistance to oxidation. St Laurent *et al.* (1990), and latterly Jensen *et al*. (1999) and Weiss and Wyatt (2003), noted that there was no further increase in α-tocopherol levels in milk above a certain level of dietary vitamin E. Weiss and Wyatt (2003) reported maximum concentration and daily secretion rate of α-tocopherol in milk at a daily intake of approximately 5000 mg of dietary α-tocopherol (from supplemental and dietary sources) and they suggested that this limit was due to the capacity of the animal to absorb α-tocopherol from the gut or by the capacity of the plasma lipoproteins to carry α-tocopherol.

6.9 Selenium

In relation to the optimum concentration of Se in cow's milk, there are no clear guidelines. Previous researchers have suggested the Se content of cow's milk for human consumption should be at least 20 µg/l, which could potentially supply >10% of the human requirement for Se in the diet.

The Se content of cow's milk can vary substantially, depending on levels in soil and water and cereals (Muñiz-Naveiro *et al*., 2005). Muniz-Naveiro *et al*. (2005) reported that the Se concentration in cow's milk varies with origin, feed, soil conditions and season. The bioavailability of Se in a food source is also another component to consider.

6.9.1 Effect of season

The concentration of Se in cow's milk over the four seasons in Australia is given in Table 11.

Table 11 Concentration of Se in cow's milk over the four seasons in Australia (µg/kg milk)

Se	Mean	s.e.	Min.	Max.	Autumn	Winter	Spring	Summer
	12.6	0.48	1.7	37.1	15.9_{c}	8.4_{a}	11.4_{ab}	13.0_{bc}

Values with different subscripts are significantly different at the $P < 0.05$ level

(Walker *et al*., 2006)

Tinggi *et al*. (2001) examined milk samples from 24 selected dairy farms in Queensland, Australia, where samples were analysed for Se during summer, winter, autumn and spring. Selenium levels of 23.8 µg/l, 20.9 µg/l, 20.7 µg/l and 20.6 µg/l were recorded in each season respectively and a correlation between season and region was noted, in that high Se levels in summer milk were related to high regional Se levels.

6.9.2 Effect of fat content of milk

Differences in the Se content of whole milk and skimmed milk samples sourced from Spanish supermarkets were recorded by Muñiz-Naveiro *et al*. (2005). Selenium levels in whole milk varied between 12.2-21.0 µg/l and 8.5-17.2 µg/l in skimmed milk.

6.9.3 Effect of diet/region

Soil Se levels vary around the world and therefore diet/region will have an impact on milk Se levels. For example, Tinggi *et al*. (2001) outlined the levels of Se in cow's milk in various countries, ranging from low levels of 7.9 µg/l in Finland to 32-138 µg/l in southern USA (Table 12). In Scandinavian countries, the Se content of the soil is particularly low (Cottrill and Givens, 2003). Even within a country, the Se levels in cow's milk can vary. For example, in Australian herds, Se levels ranged from 20-40 µg/kg (McNaughton and Marks, 2002) but can vary according to the source of the cow's diet. In a review of the effect of nutrition and management on the composition of milk (Walker *et al*., 2004), it was stated that Se levels in milk varied depending on the concentration of Se in the animal's diet. A study of Se levels in milk from 24 dairy farms in Northern Victoria demonstrated differing Se levels ranging from 7-37 µg/kg, depending on the feed source (Walker *et al*., 2003). Variation in the Se content of Australian forages was reported in a study by Caple *et al.* (1980), with levels ranging from 10->50 µg Se/kg and likewise with cereals, with Se levels ranging from 1-117 µg/kg for wheat and 10-488 µg/kg for lupin (White *et al*., 1981). A study by Heys and Hill (1984) to measure Se levels in cereals and herbage grown on farms in England and Wales indicated low levels of Se in 39% of silage samples and 60% of grain (<0.03 mg/kg DM).

Selenium content varied slightly among the grain samples (ranging from 0.025 mg/kg to 0.032 mg/kg), with the greatest variation reported between silage samples (ranging from 0.045 mg/kg to 0.071 mg/kg). This variation was attributed to silage samples from one particular area in Cambridge, demonstrating a regional effect.

Table 12 Se content of cow's milk in various countries

Country	Se (µg/l)	Year reported
Finland	7.9	1981
Germany	23.2	1978
Norway	10.1	1983
Netherlands (winter)	16.4	1989
Netherlands (summer)	10.1	1989
Belgium (summer)	11.3	1988
Belgium (winter)	17.3	1988
Poland	10.5	1992
UK	15.0	1995
Canada	28.0	1980
USA (Ohio)	8.0	1979
USA (South Dakota)	32-138	1984
New Zealand	2.9-9.7	1973
Australia	15.8	1983

(Tinggi *et al*., 2001)

The origin of foods and feedstuffs can also affect levels of Se in milk. For example, Murphy and Cashman (2001) investigated the Se content of a range of commonly consumed Irish foods, with particular reference to breads and flours. Wheat imported from Canada and North America, used in UK bread-making was Se rich, however as a consequence of EU policies these imports have been restricted and substituted with Se poor European wheat varieties.

6.9.4 Form of selenium in cow's diet

Givens *et al.* (2004) investigated the relationship between dietary supplementation with Se and the Se content of cow's milk. Three different treatments were offered to 90 Holstein cows over an 8-week period namely, sodium selenite (Selenium Metasolate™), a chelated Se product and a selenium yeast, Sel-Plex™ at 3 different levels, 0.38, 0.76 and 1.14 mg/kg. Increases in the level of milk Se were observed in all 3 treatments but cows offered Sel-Plex™ had the greatest increase, >65 µg/l. Offering Sel-Plex™ at the lowest dietary concentration resulted in milk Se increasing to a level of 28 µg/l. If the Se content of milk was increased to this level, with current daily UK milk consumption averaging 260 ml/day, this could increase the level of Se intake from milk by an extra 7.3 µg/day, which would improve daily Se intake by 11% for men and 14% for women. Table 13 illustrates the influence of Se yeast and sodium selenite on milk selenium concentration.

Table 13 Dry matter intake, milk protein and fat content, Se intake on diets supplemented with different sources and levels of Se

Treatment Levels of Se	Dry Matter Intake (kg/d)	Se intake (mg/d)	Milk Se content (µg/l)	Milk fat (g/kg total fat)	Milk protein (g/kg total protein)
Inorganic Sodium Selenite					
Low	21.6	9.4	13.6	40.4	34.8
Medium	19.5	12.6	15.9	42.9	35.6
High	20.4	15.8	18.2	41.0	34.9
Organic Sel-Plex (Yeast)					
Low	20.8	8.5	28.4	40.7	34.9
Medium	20.8	15.3	68.2	41.2	35.1
High	20.5	22.1	96.6	44.8	35.2
Chelated inorganic Se amino acid complex					
Low	19.9	10.3	13.6	40.7	35.1
Medium	20.2	15.6	13.6	40.8	35.6
High	20.5	19.8	20.5	40.3	34.9
s.e.	0.41	0.03		1.37	0.31
P-value					
Source	NS	***	***	NS	NS
Level	NS	***	***	NS	NS
Source X Level	*	***	***	NS	NS

NS=Not significant; *P < 0.05; ***P < 0.001

(Ministry of Agriculture, Fisheries and Food (MAFF), 2001)

An *in vitro* method for comparing the bioavailability of Se in cow's milk after the use of different diets was developed by Muñiz-Naveiro *et al*. (2006). Selenium was added to a forage diet in the form of organic selenium (selenised yeast) and sodium selenite. Results demonstrated that it was possible to enrich cow's milk with Se at different concentrations and that the bioavailability of Se was greater in the diet supplemented with organic Se, with levels of 0.4 and 0.5 µg Se/g as opposed to the inorganic sodium selenite with levels of 0.2 to 0.3 µg Se/g. Concerns regarding the addition of Se to the diet of humans and farm animals have been raised in the past. Rayman (2004) reviewed the use of high Se yeast as a supplement in cow diets and reported that it was more effective than inorganic Se in increasing the Se content of milk and cheese. An information paper from the advisory committee on animal feedstuffs (ACAF/05/12) detailed concerns relating to several unauthorised organic Se proprietary products which were subsequently withdrawn from sale within the EU as only inorganic forms, sodium selenite and sodium selenate were authorised as Se element feed additives. However, the organic Se animal feed supplement, Sel-Plex®, (a selenised yeast) produced by Alltech (UK) Ltd, has now been authorised for use in the European Union (EU regulation 1750/2006).

The Gardiner Foundation, Australia, are currently funding research into developing the methodology for the production of high Se milk (http://www.gardinerfoundation.com.au/press_releases/MP4-009%20PDoyle.pdf)

6.9.5 Biofortification of food crops with Selenium

Researchers at DEFRA are examining methods to fortify Se levels in wheat by agronomic biofortification (via the use of Se fertilizers) and plant genotype selection (Broadley *et al*., 2006).

6.9.6 Breed of cow

No data were found in the literature regarding the effect of breed on Se levels in milk.

6.10 *n*-3 Polyunsaturated fatty acids/CLA

The main fatty acids found in milk are presented in Table 14.

Table 14 Fatty acid profile of milk fat

Fatty acid	Proportion (g/100 g fatty acids)
C4:0 (Butyric*)	3
C6:0 (Caproic*)	2
C8:0 (Caprylic*)	1
C10:0 (Capric*)	3
C12:0 (Lauric*)	4
C14:0 (Myristic*)	12
C16:0 (Palmitic*)	26
C18:0 (Stearic*)	11
C18:1*cis*	28
C18:1*trans*	0.4
C18:2 (Linoleic)	2
C18:3 (Linolenic)	1

*Saturated fatty acid

(Walstra and Jenness, 1984)

6.10.1 Effect of diet

In an extensive review of the literature by Woods *et al*. (2005), the effect of offering *n*-3 rich diets to dairy cows on the subsequent fatty acid profile of milk was examined and a summary of results are given in Table 15.

Table 15 Effect of diet on the fatty acid profile of milk

Feed	Main fatty acid	Milk
Linseed	ALA	↑PUFA, ↓fat + protein
Hemp oil	ALA	N/A
Chia seed	ALA	N/A
Marine Algae	*n*-3	↑CLA, ↑DHA, ↓intake, ↓milk fat
Fish oil/meal (unprotected)	*n*-3, EPA, DHA	↑CLA, ↓intake, ↓milk yield, ↑DHA, ↑*n*-3
Lupin	C18:1	↓*n*-6:*n*-3
Canola/rape meal/seed	*n*-6, *n*-3	OSR ↑C18:1, ↓C16:0, ↑CLA
Soya	*n*-6	↑CLA, ↑C18:2, ↓SFA
Grass	ALA	↑CLA, Small ↑ALA
Red clover	ALA	↑ALA

ALA=α-linolenic acid; PUFA=Polyunsaturated fatty acid; CLA=Conjugated linoleic acid; EPA=Eicosapentaenoic acid; DHA=Docosahexaenoic acid; SFA=Saturated fatty acid

(Woods *et al*., 2005)

The effects of various forage treatments (including offering fresh and conserved forages, varying forage inclusion ratios, alpine and lowland grass or including different plant oils) on the fatty acid profile of milk was extensively reviewed by Dewhurst *et al*. (2006). These authors concluded that the most substantive effects were recorded with dietary inclusion of fresh leafy grass, improving the PUFA in milk and the beneficial effect of red clover silage for increasing C18:3 *n*-3 in milk fat.

Other papers published since the review by Woods *et al*. (2005) include that of Elgersma *et al*. (2006), a review paper to examine the fatty acids in milk produced in different regions of the world, different seasons of the year and under different feeding systems. These authors concluded that milk from cows offered fresh green forage, especially those offered grazed grass, had a much higher proportion of unsaturated fatty acids:saturated fatty acids, with more PUFA and more CLA (C18:2 *cis*-9 *trans*-11 in particular) than cows offered silage. Ellen *et al*. (2003) also found that milk had higher levels of the beneficial unsaturated fatty acids when cows were at grass as opposed to being housed. These authors also commented how oils such as peanut, sunflower or linseed could be offered to cows at housing to increase the proportion of beneficial fatty acids in the milk. Ellen *et al*. (2003) conducted a study to examine the fatty acid profile of milk samples drawn via a mobile milk collection system each month in four regions of the Netherlands, from August 2001 to July 2002. Data from this study demonstrated that vaccenic and *cis*-9, *trans*-11 CLA levels were twice as high from the milk of cows at pasture as opposed to those housed indoors. Bargo *et al*. (2006) also concluded that supplementing total mixed rations with pasture increased the content of CLA in milk.

6.10.2 Effect of Diet/Season

New Zealand researchers examined the seasonal and lactational influences on bovine milk composition (Auldist *et al*., 1998). Proportions of CLA were significantly affected by stage of lactation and season, with highest values being reported in late spring. A similar trend was given when the proportion of polyunsaturates in milk was examined (Table 16).

Table 16 Proportion of CLA and PUFA in milk from cows in early, mid and late lactation, in spring, summer, autumn and winter

	CLA	**PUFA**
Spring		
Early	0.97	4.29
Mid	1.11	4.27
Late	1.27	4.70
Summer		
Early	0.63	3.31
Mid	0.74	3.30
Late	0.79	3.52
Autumn		
Early	0.95	3.96
Mid	0.89	3.95
Late	0.94	4.44
Winter		
Early	0.60	3.90
Mid	0.94	4.08
Late	0.87	4.08
Significance		
Stage of Lactation	**	**
Season	**	**

(Auldist *et al*., 1998)

This research is also in agreement with that of Castillo *et al*. (2006), who concluded that the majority of medium and long chain fatty acids in milk were higher in cows offered spring high alfalfa diets compared to those offered winter low alfalfa diets (Table 17).

Table 17 Seasonal variation of milk fatty acids as a proportion of the total milk fat (g/kg) in 8 alfalfa-grazing farms in Argentina

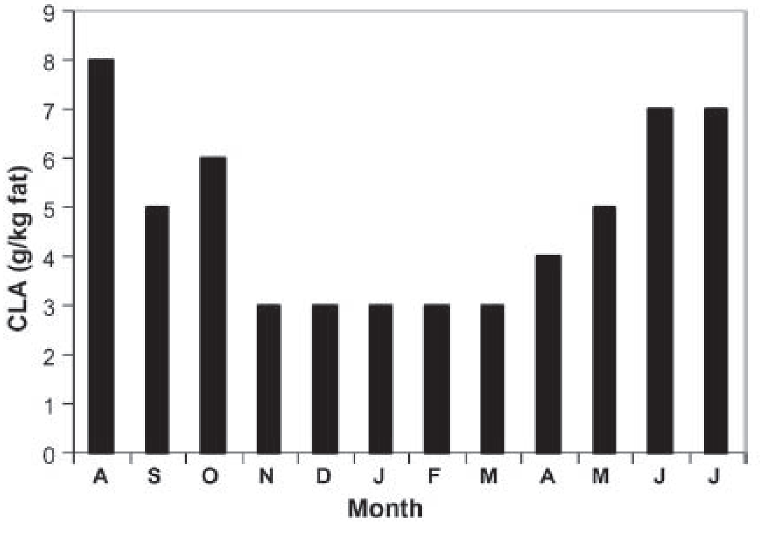
Fatty acid	**Winter**	**Spring**	**Summer**	**Autumn**	**s.e.m.**	***P*-value**
C16:0	255.0^{ab}	239.0^{b}	267.0^{a}	255.2^{ab}	5.08	**
C18:1 *cis*	194.5	205.4	190.6	191.4	4.36	NS
CLA	12.8	14.6	12.8	14.2	0.70	NS
C18:2	23.0^{a}	23.0^{a}	14.1^{b}	13.5^{b}	0.83	**

Within rows, means with different superscripts differ

(Castillo *et al*., 2006)

In a review by Elgersma *et al*. (2006) to assess the effect of season on the fatty acid profile of milk, a seasonal effect on milk CLA concentration was demonstrated (Figure 1) (modified from Ellen *et al*. (2003)).

Figure 1 Effect of season on milk CLA concentration (modified from Ellen *et al*. (2003))



(Elgersma *et al*., 2006)

Researchers in the UK also examined the effect of season on the fatty acid composition of milk and concluded that the CLA content of milk in May, June and July was significantly higher ($P < 0.05$) than for all other months, being 1.5 g CLA/100 g fatty acid methyl ester (FAME), compared with a mean of 0.77 g/100 g for the other months (Lock and Garnsworthy, 2003). During the winter months, cows were offered a total mixed ration of grass silage and maize silage, brewers grains, cereals, soya and dairy concentrate, whilst the cows were offered grass during the summer months, with increased levels of buffer feeding as the summer progressed. A summary of the main findings from this study is given in Table 18.

Table 18 Fatty acid profile of milk (FAME g/100g) from a commercial dairy herd over 3 years

Fatty acid	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	s.e.d.	Sig
***Cis*-9 C18:1**	21.4	25.9	23.6	24.5	22.8	23.1	23.4	23.4	24.7	23.9	23.5	0.90	***
***Cis*-9, *trans*-11 C18:2**	0.9	0.7	0.9	1.4	1.7	1.4	0.9	1.0	0.6	0.6	0.6	0.09	***
***Cis* 9,12,5 C18:3**	1.1	0.9	1.1	0.8	1.0	1.0	1.1	1.2	0.9	0.9	1.0	0.04	***
Total PUFA	5.6	4.8	5.5	4.6	5.6	6.4	5.9	6.0	4.9	4.5	5.0	0.24	***

(Lock and Garnsworthy, 2003)

6.10.3 Animal and breed effect

The fatty acid profile of summer milk from cows offered pasture was influenced by breed, with a higher concentration of CLA in Polish red cows compared with black and white cows (11.9 g/kg and 9.4 g/kg respectively) (Zegarska *et al*., 2001). American studies have demonstrated a breed effect (Holstein, Jersey, Brown Swiss) when the fatty acid levels of cows offered increasing levels of dietary fat were examined (Carroll *et al*., 2006). Milk fat from Jersey cows had higher proportions of short-chain and medium-chain fatty acids and Brown Swiss cows had the highest proportion of C18:1 in the milk fat (Table 19). Breed of cow had no effect on the concentrations of C18:0, C18:2 or C18:3.

Table 19 Fatty acid levels (g/100 g fat) of Holstein, Jersey and Brown Swiss cows offered increasing levels of dietary fat

Fatty acid	**Diet (g/kg fat added)**					**Significance**	
	0	**15**	**30**	**45**	**s.e.**	**Breed**	**Diet**
C16:0							
Holstein	37.76	32.34	28.09	26.61	0.23	0.57	<0.01
Jersey	35.61	31.98	27.80	27.24			
Brown Swiss	35.44	30.67	27.73	26.45			
C18:1 *cis* 9 and *cis* 10							
Holstein	13.36	18.60	22.99	24.95	0.29	0.01	<0.01
Jersey	14.52	17.16	20.94	22.47			
Brown Swiss	16.08	22.11	26.77	27.95			
C18:2 *cis* 9 and *trans* 11							
Holstein	0.33	0.46	0.67	0.81	0.021	0.18	<0.01
Jersey	0.29	0.35	0.52	0.67			
Brown Swiss	0.34	0.49	0.63	0.80			
C18:3							
Holstein	0.81	0.64	0.60	0.54	0.014	0.59	<0.01
Jersey	0.84	0.66	0.59	0.54			
Brown Swiss	0.74	0.62	0.57	0.51			

(Carroll *et al*., 2006)

Belgian researchers also reported variation in the fatty acid content of milk within and across breeds, when dual purpose Belgian Blue, Holstein-Friesian, Jersey, Montbelliarde and non-Holstein Meuse Rhine-Yssel type Red and White were examined (Soyeurt *et al*., 2006). The most notable difference in the milk fatty acid content was found between Jersey and Holstein cows, where the fatty acid profile of the milk was significantly different between the two breeds, with the exception of C16:1 *cis*-9 (P = 0.23).

6.11 α-Lactalbumin and lactoferrin

6.11.1 Effect of nutrition and management

It is important to distinguish between those factors that affect protein content (percentage) in milk, those that affect protein yield (kg protein/day) and those that affect protein composition (protein fractions). Dietary changes that increase milk and protein yields may cause negative effects on protein content. DePeters and Cant (1992) reviewed opportunities to modify the nitrogen composition of milk and more recently Walker *et al.* (2004) examined the effect of nutritional and management factors on milk fat and protein content. The objective of most studies and for producers is to increase protein content while maintaining or increasing milk yield.

Factors such as mastitis, high somatic cell count, advanced parity, stage of lactation and a low level of milk production can alter the ratio of casein:serum protein (DePeters and Cant, 1992) and affect the physical functional properties of the milk (Dalgleish, 1993). Alpha-lactalbumin, β-lactoglobulin, lactoferrin, lactoperoxidase as well as the casein proteins are produced in the mammary gland, whereas the immunoglobulins, bovine serum albumen and plasmin/plasminogen are derived via circulation. Genetic variants exist for the major protein fractions derived from the mammary gland however generally α-lactalbumin in milk from European cattle is monomorphic and only the α-lactalbumin B allele is found. Cows with the B allele are associated with a higher percentage of protein and fat in milk while those with the A allele have higher yields of milk, protein and fat (Martin *et al*., 2002). Both absolute concentration in milk and proportion of α-lactalbumin as a proportion of total protein decreased during lactation (Ostersen *et al*., 1997). Nutrition however, appears to have little effect on the relative rates at which the major proteins are synthesised by mammary secretory cells (MacRae *et al*., 2000).

6.12 Vitamin C

6.12.1 Effect of Season

Season can influence the concentration of ascorbic acid in milk, with levels being almost two fold higher in March or August than October in unpasteurised milk (20-27 mg/l and 12 mg/l respectively) (Andersson and Öste, 1992).

7 Conclusions

It is well recognised that milk is a nutritious, low fat food for humans (Huth *et al*., 2006; Bauman *et al*., 2006). The British Nutrition Foundation (2003) recommend 2-3 servings of milk and milk products daily as part of a healthy diet and dietary guidelines for the United States recommend 3 servings of milk and milk products daily to reduce the risk of low bone mass and to contribute nutrients that may assist with other health attributes (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2005). Historically, the more common method employed to alter milk fat composition was via altering the nutrition of the animal. In a review of the major scientific advances in this area over the past 25 years (Jenkins and McGuire, 2006), it was reported that the important nutritional factors were forage to concentrate ratio, amount of dietary protein, source of dietary protein and the amount of fat in the diet. Based on the evidence presented in this report, the role of milk nutrients in meeting the nutritional requirements of humans are well established. The major methods whereby the concentration of these beneficial nutrients in milk can be enhanced are by dietary modification and season of production. For example, the concentration of calcium in milk is influenced by season, as are the concentrations of the trace elements such as phosphorus, magnesium and zinc. Diet and region can alter Se levels in milk significantly and the fatty acid profile of milk fat can also be enhanced via dietary modification.

Previous goals of agricultural research have focused on improving yield and efficiency of production of food products, but as modern society places a greater emphasis on the health attributes of food, a greater focus has been placed on improving the nutrient profile of food (Bauman *et al*., 2006). It is also worth noting that Jenkins and McGuire (2006) recognised that the ability to control milk composition via nutrition originated largely as a result of contributions from scientific studies of the entire animal, involving both practical feeding studies and cellular work on mammary tissue metabolism.

The composition of milk fat can be altered by dietary modification but milk protein is less responsive to changes in the animal’s diet (Jenkins and McGuire, 2006). The mammary gland is capable of producing a large amount of proteins and previous research has demonstrated that milk contains specific proteins, peptides and fatty acids that are “bioactive”, in that they provide benefits to human health over and above those associated with traditional nutrients. Milk may be regarded as a functional food (Bauman *et al*., 2006). The role of bioactive food components has been highlighted as a key area for future research to address major health threats to humans (Frontiers in Agricultural Research: Food, Health, Environment, and Communities, 2003). Whilst examining future opportunities to modify milk composition, Jenkins and McGuire (2006) also highlighted the future role of milk as a nutraceutical to improve human health and to address clinical diseases such as obesity, osteoporosis or lactose intolerance.

As we move through the 21st Century, an era where genetic engineering is more prevalent, the use of animals as “bioreactors” for “pharming” purposes will continue to gain recognition, in particular the “pharming” of beneficial proteins not normally present in milk (Bauman *et al*., 2006). Such research will require input from animal nutritionists and food scientists, in addition to significant input from the processing sector as the dairy industry has not yet explored the mammary gland as a vehicle for producing novel products to improve human health.

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