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The micronutrient content of bovine whole milk powder: Influence of pasture feeding and season

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A combination of techniques was used to investigate the content of water-soluble vitamins (thiamin, riboflavin, niacinamide, pyridoxal, ascorbic acid, choline, inositol, biotin, pantothenate, folate and vitamin B_{12}) and fat-soluble vitamins (A, E and β-carotene) in spray-dried whole milk across an entire production season.

The exclusive pasture feeding husbandry practised in the collection region, coupled with a confined calving period, has facilitated an interpretation of temporal variations in nutrient content, undistorted by the influence of variable feeding and staggered lactation regimes employed in previous surveys of this type.

This information is intended to extend the current global information regarding milk and its products.

INTRODUCTION

Milk is a principal component of the human diet, and this is recognition of its broad nutritional contribution, which includes all known vitamins and essential micronutrients. As a highly versatile yet perishable commodity, cow's milk is commonly converted to powder form in order to facilitate storage and trade and thereby extend its use as a food for community health. This practice widely incorporates supplementation with certain micronutrients, notably vitamins A and D, and more extensively in infant formulations based on bovine milk.

There have been relatively few comprehensive surveys describing the influence of environmental factors on the micronutrient composition of cow's milk, although earlier studies have been summarised in Hartman & Dryden (1974), Renner (1983) and Cremin & Power (1985). More recently, pasteurised liquid milk has been investigated as a function of season, region and breed factors as well as under storage (Scott *et al.*, 1984; Laukkanen *et al.*, 1988). Certain milk products, including powders, were also studied in relation to vitamin levels in fluid milk (Scott & Bishop, 1986). The influence of thermal processing protocols and storage on certain nutrients has been reported in both human (Goldsmith *et al.*, 1983) and cow's milk (Oamen *et al.*, 1989). Other studies have emphasised the effects of storage on the vitamin content of milk powders held under adverse conditions (Ford *et al.*, 1983; Kneifel, 1989).

A common feature of these northern hemisphere studies is the origin of raw milk from herds sustained on an alternating basis, by pasture and dry feeding regimes and where calving is seasonally less restricted. This study, therefore, aimed at investigating possible temporal influences in milks derived from cows grazed extensively and exclusively on pasture and where herd lactation commences in early spring, thereby eliminating the superimposition of such variable factors. Further, there seemed the need to complement previous studies with equivalent data derived from milk produced by herds subject to such different dairy husbandry practice.

Despite the potential vulnerability of certain nutrients to heat processing and the somewhat conflicting information on this issue, the present study has surveyed dried whole milk, in which form milk is widely traded internationally and for which a nutritional data base is commonly required. Significantly, modern spray-drying has, in fact, been shown to cause relatively minor losses of vitamins compared to either high temperature processing or storage, with the consequence that milk powder composition reflects, with reasonable accuracy, the contributions of raw milk (Causeret, 1977; Renner, 1983; Laukkanen *et al.*, 1988; Scott & Bishop, 1986; Oamen *et al.*, 1989). In addition, the compositional and structural stability of dry milk, maintained under subzero conditions, facilitates a consolidation of analysis protocol, thereby benefiting interpretation of data.

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MATERIALS AND METHODS

Milk powder samples

Whole milk powder samples were collected predominantly from a central processing site during the period August 1990 to May 1991, and were manufactured from pooled, pasteurised milk collected almost exclusively from Friesian-cross herds, subject to standard dairy practice. The product was typical of a low- to medium-heat schedule following standardisation to constant fat level, and was commonly supplemented with vitamins A (as the acetate ester) and D_3. Collection frequency was twice monthly, with a representative composite subsampling regime. On receipt, samples were subsampled for individual nutrient assay and stored deep frozen at −20°C in sealed foil until analysed.

One sample of whole milk powder from a different source was utilised as an 'in-house' reference control with each analysis set, in order to generate statistical precision parameters for each analyte and monitor between-run performance.

Analysis scope

Proximate analyses (fat, protein, moisture and ash) were performed on all samples in order to facilitate, if required, normalisation of micronutrient data on either a 'milk solids non-fat' or 'fat' basis.

Samples were subsequently analysed for fat-solubles (vitamin A, vitamin E and β-carotene) and water-solubles (riboflavin, thiamin, niacinamide, pyridoxal, ascorbic acid, folate, pantothenate, biotin, B_{12}, choline and inositol).

Analytical methods

—Standard IDF methods were used in the determination of fat, protein, moisture and ash.

—Vitamin A as retinyl palmitate was measured as the total of the 13, *cis*- and all *trans*-ester after solvent extraction of lipid and subsequent normal phase HPLC (Woollard & Blott, 1986). This is a selective method, capable of distinguishing the intact endogenous vitamer from the acetate form in which it is commonly added.

—β-Carotene was estimated spectrophotometrically following isolation of the non-saponifiable fraction (Indyk, 1987)

—Vitamin E as α-tocopherol was measured by reversed-phase HPLC coupled with fluorescence detection of the non-saponifiable extract (Indyk, 1988).

—Vitamin C analysis was performed using redox colorimetric titration with 2,6-dichlorophenol indophenol and determined as reduced ascorbic acid only.

—Thiamin, riboflavin, niacinamide and pyridoxal were estimated by reversed-phase ion-pair HPLC following reconstitution, enzymic digestion with acid phosphatase (Sigma) and clarase (Miles Lab., Australia), protein removal with trichloroacetic acid and clarification. The technique was a modification of that published by Woollard (1984) and incorporated sodium dioctylsulphosuccinate as ion-pair reagent in a methanol : water buffered phase. Fluorescence detection at 390 nm (290 nm excitation) was employed for pyridoxal while UV at 436 nm was utilised for riboflavin, with thiamin and niacinamide viewed at 254 nm.

—Biotin and 'free' pantothenate were assayed with *Lactobacillus plantarum* (ATCC 8014) as described by Bell (1974), Scheiner (1985) and Wyse *et al.* (1985).

—Total folate was extracted in the presence of antioxidant and measured with *L. casei* (ATCC 7469) after incubation with conjugase as described by Bell (1974) and Keagy (1985).

—Vitamin B_{12} estimation as cyanocobalamin was achieved with *L. leichmannii* (ATCC 7830) as described by Chin (1985), Andersson *et al.* (1990) and Kamei *et al.* (1989).

—Myoinositol was assayed, subsequent to hot acid hydrolysis, with *Saccharomyces uvarum* (ATCC 9080) based on the work of Baker *et al.* (1990).

—Choline was released with sequential acid hydrolysis under reflux, incubated with phospholipase D and assayed spectrophotometrically following treatment with choline oxidase (Woollard & Indyk, 1990).

Analytical precision

Repeatability and reproducibility are critical tests of the reliability of assay methods in such a study where long-term trends are in question. Overall 'between-run' variability was therefore established for each determinant in the control reference powder assayed with each sample set. Analyte levels were calculated as the mean of 'within-run' sample duplicates, except for those based on microbiological methods, where 'between-run' triplicate determination (at four serial dilution levels) was mandatory, in view of the generally higher uncertainty of these techniques.

RESULTS

Method precision parameters established for the reference control are listed in Table 1. Proximate compositional analysis revealed very little variation across the entire production season inclusive of occasional samples selected from other geographical regions. This information is consolidated for all experimental samples in Table 2.

Of principal significance in the context of this study are the minimal statistical variance values for both fat and 'milk solids not fat' in whole milk powders surveyed. Consequently, all micronutrient data reported subsequently are on an 'as-is' basis. Table 3 lists data

Table 1. Precision data ('between-run') for reference sample (values expressed per 100 g 'as-is')

Analyte	Mean	*n*	Std Dev.	CV(%)
Thiamin (mg)	0·34	12	0·02	5·9
Riboflavin (mg)	1·68	12	0·11	6·9
Niacinamide (mg)	0·80	12	0·05	6·0
Pyridoxal (mg)	0·25	12	0·01	5·3
Ascorbic acid (mg)	14·7	13	1·4	9·3
Pantothenate (free) (mg)	4·3	10	0·15	3·4
Choline (mg)	94·8	12	6·9	7·3
Inositol (mg)	27·8	9	7·8	22·0
Folate (μg)	57·3	10	9·6	16·7
Vitamin B_{12} (μg)	2·2	13	0·34	15·3
Biotin (μg)	12·7	11	1·4	10·9
α-Tocopherol (mg)	1·25	9	0·08	6·4
β-Carotene (mg)	0·40	8	0·02	5·1
Retinyl palmitate (μg)	760	6	40	5·3

obtained from the single site survey across an entire production season and Fig. 1 illustrates the individual analyte data graphically.

Relative standard deviations for most analytes across the season, were significantly higher than for the reference control, reflecting a true variance of content in experimental samples. The similarity of this parameter between reference and unknown samples in the case of inositol, folate and vitamin B_{12} may additionally reflect the recognised analytical difficulties with these particular microbiological assays, both in terms of level and culture maintenance (Walenrod & Levinton, 1971; AOAC, 1984; Chin, 1985; Favell, 1990).

Table 2. Consolidated proximate data for experimental whole milk powders (WMP) across season and region (values expressed as %)

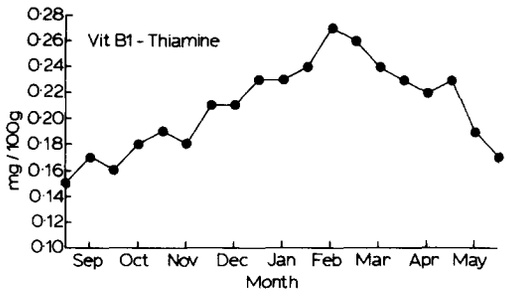
Analyte	Product	Mean	*n*	Std Dev.	CV (%)
Fat	WMP	27·3	26	0·81	2·9
Ash	WMP	5·72	26	0·17	3·0
Moisture	WMP	2·65	26	0·16	6·0
Protein	WMP	27·8	26	0·59	2·1
MSNF	WMP	70·02	26	0·83	1·2

There was a discernible and essentially systematic trend for thiamin, choline and folate, with evidence of a seasonal inflection, while the remainder of the water-soluble nutrients revealed either an absence of significant trend (riboflavin, pyridoxal, inositol, vitamin B_{12}), a shallow decline (niacinamide, pantothenate) or slight rise (ascorbic acid, biotin) across the season.

All three naturally occurring fat-soluble vitamins (retinyl palmitate, β-carotene, α-tocopherol) revealed a clear, almost coincident mid- to late-summer seasonal minimum rising to an early spring maximum. It is important to emphasise that this trend was observed and was equivalent even when expressed on a normalised fat basis and therefore reveals at least some biogenic independence from milk-fat content.

Table 3. Vitamin contents in whole milk powder across production season (expressed per 100 g 'as-is')

Date	B_1 (mg)	B_2 (mg)	B_3 (mg)	B_6 (mg)	C (mg)	Pant. (mg)	Chol. (mg)	Inos. (mg)	Fol. (μg)	B_{12} (μg)	Biot. (μg)	E (mg)	β-Car. (mg)	A (μg)
1990														
August 16	0·15	1·7	0·72	0·23	9	3·2	54	56	58	2·9	15	1·43	0·39	740
September 1	0·17	1·5	0·67	0·22	8	3·3	58	47	57	2·6	16	1·49	0·38	590
September 16	0·16	1·5	0·62	0·21	9	3·1	57	43	53	2·3	17	1·32	0·36	540
October 1	0·18	1·7	0·62	0·20	11	2·7	61	48	49	2·2	14	1·19	0·34	550
October 16	0·19	1·7	0·65	0·22	11	2·5	65	39	43	2·6	16	1·17	0·29	520
November 1	0·18	1·6	0·72	0·20	12	2·6	62	42	43	2·6	17	1·11	0·28	470
November 16	0·21	1·7	0·78	0·21	12	2·5	68	54	39	2·9	22	1·12	0·26	460
December 1	0·21	1·8	0·74	0·20	11	2·9	72	49	40	3·0	22	1·04	0·23	450
December 16	0·23	1·7	0·76	0·19	13	2·7	77	46	39	3·1	21	0·88	0·20	430
1991														
January 1	0·23	1·7	0·72	0·18	12	2·4	69	43	39	3·0	20	0·96	0·19	440
January 16	0·24	1·6	0·68	0·21	11	2·1	67	47	38	2·9	23	0·98	0·18	490
February 1	0·27	1·6	0·72	0·23	12	2·3	72	41	42	2·5	21	0·91	0·16	470
February 16	0·26	1·7	0·73	0·21	13	2·4	74	48	44	3·1	22	0·82	0·13	500
March 1	0·24	1·8	0·70	0·23	12	2·0	82	57	50	2·8	24	0·92	0·14	530
March 16	0·23	1·9	0·59	0·21	13	2·1	93	58	52	2·7	24	0·98	0·16	570
April 1	0·22	2·0	0·50	0·18	12	1·8	96	60	45	3·0	26	1·21	0·19	610
April 16	0·23	2·1	0·54	0·18	13	1·8	89	52	48	2·9	25	1·42	0·24	630
May 1	0·19	2·1	0·48	0·14	14	2·0	85	50	50	3·5	28	1·49	0·25	680
May 16	0·17	2·0	0·49	0·16	14	2·0	80	46	46	3·3	27	1·50	0·29	720
n	19	19	19	19	19	19	19	19	19	19	19	19	19	19
Mean	0·21	1·76	0·65	0·20	11·7	2·44	72·7	48·7	46·1	2·84	21·1	1·15	0·25	547
SD	0·03	0·18	0·09	0·03	1·59	0·45	12·0	5·90	5·99	0·31	4·10	0·22	0·08	91·4
CV(%)	16·1	10·4	14·3	13·5	13·8	18·3	16·5	12·1	13·0	11·3	19·5	19·3	32·2	16·7



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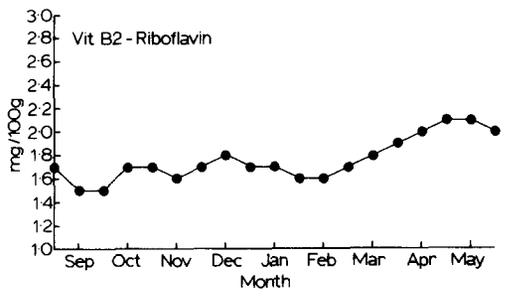

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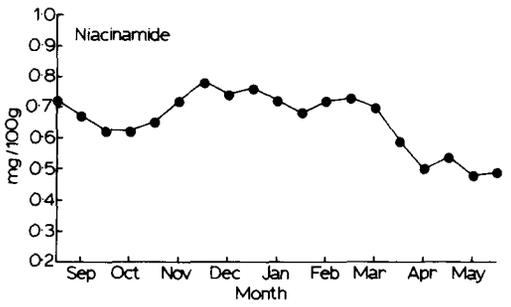

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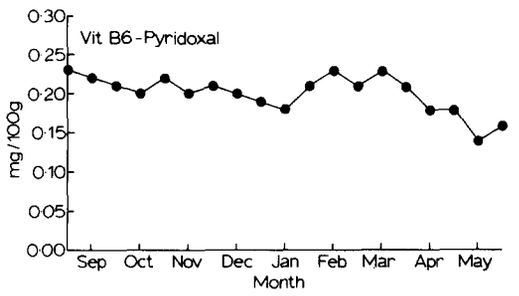

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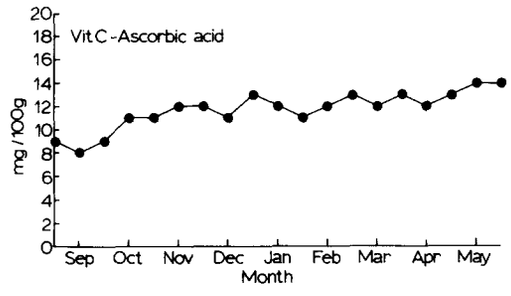

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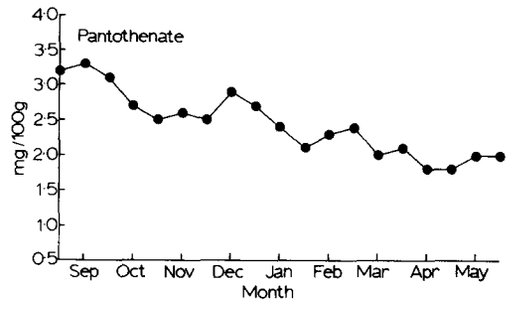

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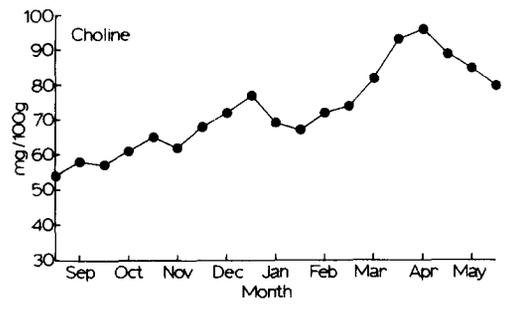

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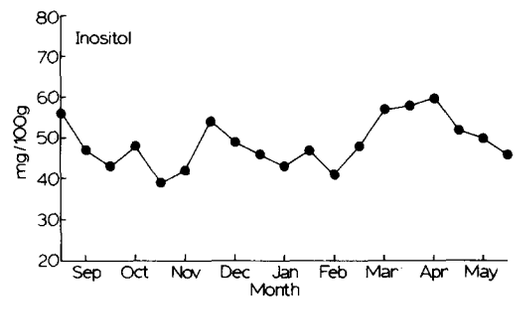

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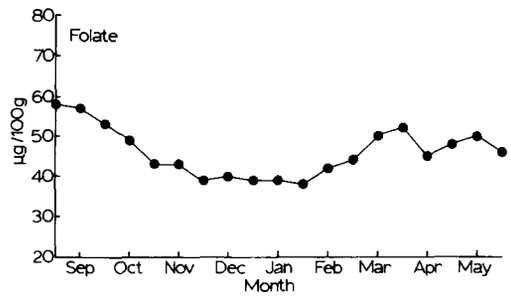

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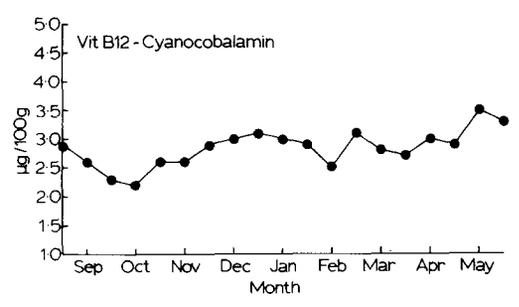

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Fig. 1—parts 1–10

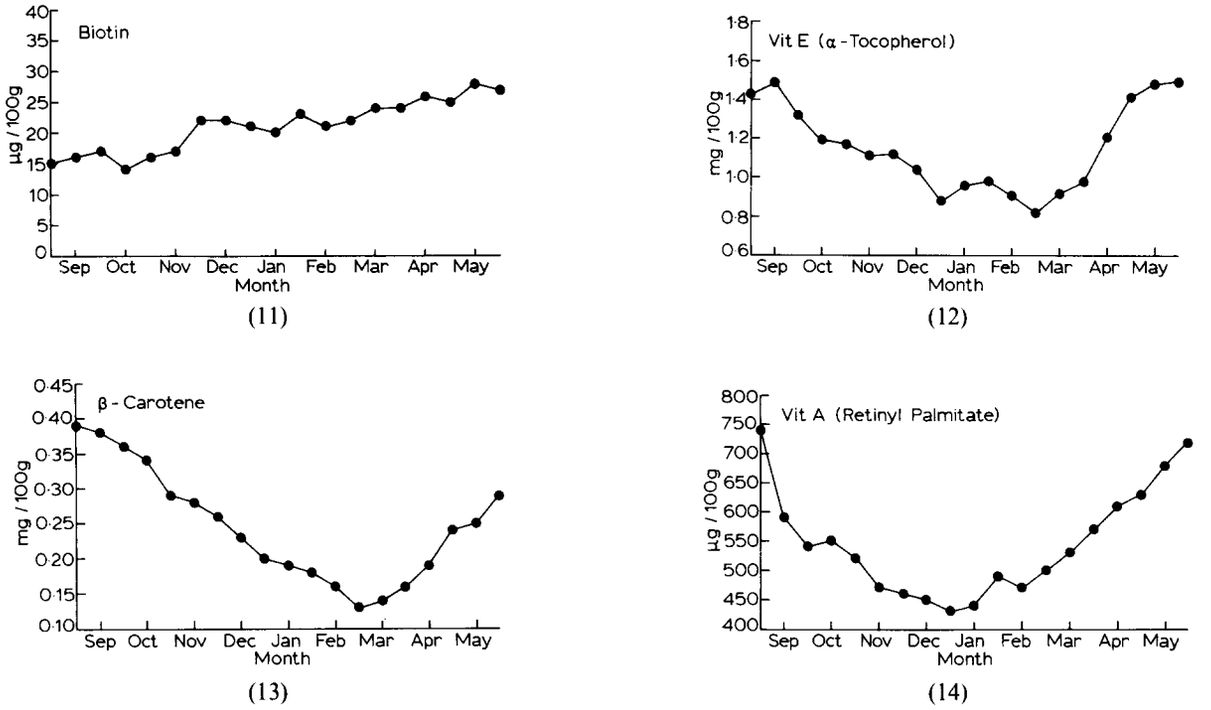

Fig. 1—parts 11–14

Fig. 1. Concentration of individual vitamins in spray-dried whole milk across 1990–91 season.

Table 4 compares the range of experimental data obtained presently, against those reported in the literature. The latter data are expressed on a solids basis, as several studies cited report on raw or pasteurised milk only.

Occasional random samples were additionally surveyed from other geographical regions within New Zealand and were quantitatively consistent with the main data base. However, an insufficient sampling regime could not confidently establish similar seasonal influences in these areas (data not shown).

DISCUSSION

Milk is commonly traded internationally in the dehydrated form, and the purpose of this survey was to establish a data base complementary to those previous reports, where either fluid or stressed milk powder was the focus of study. An important attribute of this particular survey may be that potential seasonal influences are unmoderated by artefactual dietary influences, as a consequence of the exclusive pasture feeding regime generally practised in Australasia.

Table 4. Comparison with published literature data (expressed per 100 g milk solids)

Analyte	Present study	Literature	References
Thiamin (mg)	0·15–0·27	0·15–0·40	1, 2, 3, 4, 5, 6, 7, 8, 9, 19, 22
Riboflavin (mg)	1·5–2·3	0·75–1·9	1, 2, 3, 6, 7, 8, 9, 18, 19, 20, 21, 22
Niacinamide (mg)	0·45–0·78	0·30–1·00	1, 2, 3, 6, 7, 8, 9, 23
Pyridoxal (mg)	0·13–0·23	0·10–0·60	1, 2, 3, 6, 7, 8, 9, 22, 24
Ascorbic acid (mg)	8–15	1–16	1, 2, 3, 6, 7, 8, 9, 22
Pantothenate (mg)	1·8–3·3	2·1–3·9	1, 2, 3, 6, 7, 8, 9, 10, 25
Choline (mg)	54–96	40–360	1, 2, 3, 11, 12, 34
Inositol (mg)	39–60	24–320	1, 2, 3, 34
Folate (µg)	38–58	8–90	1, 2, 3, 6, 7, 8, 9, 13, 14, 22, 25
Vitamin B_{12} (µg)	2·2–3·8	1·3–5·6	1, 2, 6, 7, 8, 9, 15, 16, 22
Biotin (µg)	14–28	8–56	1, 2, 3, 6, 7, 8, 9, 17, 25
α-Tocopherol (mg)	0·88–1·50	0·16–1·50	1, 2, 3, 26, 27, 30, 31
β-Carotene (mg)	0·13–0·39	0·05–0·60	1, 2, 3, 6, 28, 29, 31, 32, 33
Vitamin A (µg)	430–740	80–800	1, 2, 3, 6, 9, 29, 30, 31, 32, 33

1. Renner, 1983; 2. Hartman & Dryden, 1974; 3. Machlin, 1991; 4. Ayi *et al.*, 1985; 5. Nicolas & Pfender, 1990; 6. Scott *et al.*, 1984; 7. Scott & Bishop, 1986; 8. Ford *et al.*, 1983; 9. Kneifel, 1989; 10. Finglas *et al.*, 1988; 11. Woollard & Indyk, 1990; 12. Lied & Braekken, 1975; 13. Martin *et al.*, 1990; 14. Holt *et al.*, 1988; 15. Andersson *et al.*, 1990; 16. Kamei *et al.*, 1989; 17. Bitsch *et al.*, 1989; 18. Russell & Vanderslice, 1992; 19. Wills *et al.*, 1985; 20. Ashoor *et al.*, 1985; 21. Ribarova *et al.*, 1987; 22. Laukkanen *et al.*, 1988; 23. Skurray, 1981; 24. Bitsch & Moller, 1989; 25. Hoppner & Lampi, 1990; 26. Syvaoja *et al.*, 1985; 27. Indyk, 1988; 28. Indyk, 1987; 29. Kirichenko, 1989; 30. Ball, 1988; McGillivray, 1956; 32. Sivell *et al.*, 1984; 33. Ollilainen *et al.*, 1989; 34. Causeret, 1977.

Appropriate analytical methodology was selected, retaining microbiological techniques for certain members of the B-complex, with HPLC employed for the majority of the remaining vitamins. These strategies are consistent with current knowledge regarding the dominant forms within milk and the recent comprehensive reviews in the area of vitamin assay of foods (Finglas & Faulks, 1987; Ball, 1988; Favell, 1990; Lumley & Lawrance, 1990; Macrae, 1990; Polesello & Rizzolo, 1990).

In all cases, measured vitamin levels were consistent with other published data, generally falling within the overall ranges reported internationally. However, the majority of cited data are typical values only, while few of the previous studies have surveyed seasonally representative samples (McGillivray, 1956; Hartman & Dryden, 1974; Renner, 1983; Scott *et al.*, 1984; Scott & Bishop, 1986) and of these, the first two are literature reviews covering earlier work. Nevertheless, the overall similarity in micronutrient levels in bovine milk between widely diverse herd origins, demonstrates consistent biochemistry within the species.

Specific discussion of vitamins and related nutrients is commonly and conveniently divided between water- and fat-soluble compounds, with the former group comprising the so-called B-complex and vitamin C, and the latter, vitamins A (including β-carotene), E, D and K.

For the water-soluble group, a maximum range of individual vitamin levels of less than approximately two-fold was observed over the season. For most, there was little evidence of any systematic seasonal trend, except for thiamin, choline and folate, which appeared to exhibit an annual inflection. It has been documented previously that rumen microflora intervene during the biogenesis of the B-complex in the ruminant and indeed, animals fed deficient diets can maintain similar circulating levels of most vitamins (Hartman & Dryden, 1974; Scott *et al.*, 1984; Cremin & Power, 1985; Machlin, 1991). Mammary uptake from plasma and secretion in milk is likely therefore to parallel this reduced dietary dependence and account for the weak seasonal correlation observed in most cases. Translocation of certain circulating plasma vitamins to the mammary gland prior to appearance in milk, may be further moderated through the intervention of appropriate binding proteins, although the role of these whey proteins in milk has not yet been fully defined. Any discernible trends in the present study may therefore more plausibly reflect the coincidence of herd parturition during early spring, with the consequence that collected milk is of equivalent lactational maturity during the production year.

The literature, however, is not always consistent and there are various reports alleging either an increase, decrease or no change in individual water-soluble vitamins across a season. The more recent studies have demonstrated only minor or negligible temporal variation for the majority (thiamin, riboflavin, niacinamide, pyridoxal, pantothenate, biotin and ascorbic acid) while significant trending has been reported for folate and B_{12} (Scott *et al.*, 1984; Scott & Bishop, 1986; Laukkanen *et al.*, 1988). These authors concluded that dry-feeding regimes maintained during the northern hemisphere winter and parturition, may mask a true seasonally-related dietary influence on rumen flora metabolism. Moreover, the absence of a seasonally-confined calving pattern will further have compounded interpretation. It is apparent that both these factors have been largely removed during the present survey, providing strong support of the evidence suggesting that any dietary impact on ruminant milk expression acts indirectly and predominantly through stimulation of biochemical events in the rumen.

Choline and inositol, while not defined strictly as vitamins, are regarded as essential nutrients, although they have not received previous attention in the literature cited above. A recent analytical method based on enzymology has facilitated the convenient estimation of choline and has previously reported a similar seasonal trend (Woollard & Indyk, 1990), while information relating to inositol appears only to be indicative of typical values. That choline levels in milk appear to show a temporal variance unlike the majority of the B-complex vitamins, may well reflect significant post-ruminal biosynthetic origins. Indeed, free choline is degraded rapidly by rumen microflora and appears in plasma through both absorption of feed choline esters and postruminal methionine metabolism limited by dietary protein (Erdman & Sharma, 1991; Machlin, 1991).

In the case of the endogenous fat-soluble vitamins studied, strong evidence for a systematic seasonal influence existed, with close coincidence of a minimum during the mid- to late summer. Unlike the nutrients of the B-complex and vitamin C, quantities of the antioxidant fat-solubles in bovine milk are reported to be principally dependent upon the dietary contribution of these compounds and milk fat content, although vitamin K is additionally generated in significant quantities within the rumen (Hartman & Dryden, 1974; Renner, 1983). Northern hemisphere studies have generally reported higher summer levels of vitamin and pro-vitamin A, associated with a change from winter dry-feeding to spring pasture grazing (Scott *et al.*, 1984; Kirichenko, 1989). The present study is consistent with previous data relating to the Australasian situation and further reveals the trend to be largely independent of fat content, with pasture maturity and quality the dominant factors (Barnicoat, 1947; Farrer *et al.*, 1949; McGillivray, 1956; McDowall & McGillivray, 1963; Larson *et al.*, 1983; Indyk, 1987). This trend and dietary dependence seems consistent also for α-tocopherol, the predominant form of vitamin E in milk, and may reveal its close association with the biological protection and utilisation of vitamin A in tissues (Hartman & Dryden, 1974; Cremin & Power, 1985; Syvaoja *et al.*, 1985). The seasonal pattern reported presently for this vitamin is particularly significant, since unlike vitamin A and β-carotene, vitamin E

reflects, through its congener distribution, the dietary contribution of supplementary winter-fed cereal crops. However, the absence of such a practice in this study, allows a more direct interpretation to be made of pasture grazing and lactation level for this nutrient.

There were few appreciable nutrient concentration differences amongst several random samples derived from other regions within New Zealand, although the fat-soluble vitamins displayed somewhat greater variability. These observations appear consistent both with other studies cited and the present interpretation regarding the generally diminished influence of diet on expression of the B-complex micronutrients in the mammary gland, since pasture contents would be expected to display some temporal variance with latitude. Vitamin C and inositol data, however, exhibited significantly higher scatter, the former possibly as a consequence of its lability and hence greater vulnerability to processing variations, and the latter conceivably due to an inherently lower analytical precision.

Finally, data obtained from the experimental dried whole milk samples were all within the literature ranges cited for either raw or pasteurised fluid milks. It may be concluded, therefore, that modern spray-drying regimes result in relatively small losses of most vitamins, in agreement with the observations of others (Renner, 1983; Scott & Bishop, 1986).

CONCLUSION

The temporal fluctuation of vitamin content in low-moisture whole milks, derived from herds maintained under dairy management practices different from those in the northern hemisphere, has been investigated. This has facilitated conclusions to be made regarding seasonal factors, isolated from the distorting influence of dietary and lactational variables that have complicated earlier studies. Observations of minimal variation only for most of the water-soluble vitamins provide additional evidence for the view that both microbial biochemistry in the rumen and milk maturity, rather than exogenous diet, are probably controlling criteria during ruminant lactation. Significant seasonality amongst the fat-soluble group, however, concurs with the conclusions of others regarding the positive influence of diet amongst these nutrients.

In this context, it seems noteworthy that little has been reported concerning the dynamics of vitamin uptake from blood across the basal membranes of the mammary gland. Arterio-venous concentration difference measurements have been successfully utilised during investigation of the biosynthesis of several major milk constituents and, as vitamin methodologies advance, such an approach may well benefit our understanding of the specific migration of circulating micronutrients prior to secretion in milk.

There is no evidence of significant regional variation in overall vitamin content, nor of appreciable losses in the conversion of fluid to dried whole milk during spray drying. The information reported in this survey (including new indicative values for inositol and choline), should assist in the standardisation of bovine milk-based infant formulae and complement the steadily accumulating global data base regarding dairy products.

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