FACTORS AFFECTING THE VITAMIN A AND β-CAROTENE CONTENTS OF MILK

A Thesis

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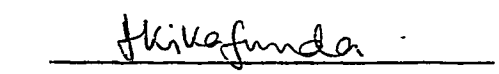

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Saskatoon, Saskatchewan

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This thesis is dedicated to my parents,

my husband, Joseph, and my sister, Addy.

ABSTRACT

The factors affecting the vitamin A and β-carotene contents of milk were investigated. A series of short- and long-term experiments were conducted using the University of Saskatchewan dairy herd and Saskatoon commercial fluid milk, respectively.

The vitamin A potency of the milk was significantly influenced by cow-to-cow variations when the experimental cows were receiving high levels (87,600 IU/kg feed) of vitamin A supplementation. The day-to-day variations significantly affected the vitamin A content of milk when early lactation cows received low levels (8,200 IU/kg feed) of vitamin A supplementation. The milk of cows in late lactation contained more vitamin A per 100 ml but the milk of cows in early lactation contained more vitamin A per gm butter fat.

Pronounced seasonal variations were observed in Saskatoon market milks. The spring milk contained 28.87%, 20.09%, and 8.33% more vitamin A potency than that of winter, fall and summer milk, respectively. Overall, spring and summer milk had significantly more vitamin A (1.17 times) than that of winter and fall milk. Supplementation of feeds with about 10 times the RDA of vitamin A increased the vitamin A content of the milk by 24% but did not affect either the milk yield or the B.F.% of the milk. Dietary hay suppressed, but dietary silage promoted, the recovery of dietary vitamin A in milk of cows fed the above forages, each supplemented with 250,000 and 600,000 IU vitamin A/cow/day. High levels of vitamin A intake depressed the milk and blood levels of β-carotene. Dietary levels of the vitamin A did not

affect the concentration of the vitamin in the blood of the experimental animals.

Pasteurization of milk had no deleterious effects on the vitamin A potency of milk. Skimming significantly reduced the vitamin A content of whole milk by 85.0% and 26.5% for skim and 2% B.F. milk, respectively. Fortification, at the processing plant, increased the vitamin A content of the low fat milk to above average levels. Maximal photodegradation of vitamin A occurred between 340 to 440 nm. β-carotene significantly protected vitamin A from photodegradation. Storage stability of vitamin A in processed milks (skim, 2% B.F. and homogenized milk) was found to be much less than that of whole, raw milk.

The results from the study indicated that the vitamin A potency of milk can be optimized by fortification of winter feeds, particularly silage, with vitamin A. Once the milk has been produced, the vitamin A levels can be optimized by proper fortification of low fat milk and by proper packaging and storage of processed milk.

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LIST OF ABBREVIATIONS

B.F.	Butter fat
C.P.	Crude protein
DM	Dry matter
DMP	Dry milk powder
FAO	Food Agriculture Organization
IU	International Unit
NRC	National Research Council
PA	Prealbumin
PCM	Protein Calorie Malnutrition
RABP	Retinoic acid binding protein
RBP	Retinol binding protein
RDA	Recommended daily allowance
RE	Retinol Equivalents
TFA	Trifluoro acetic acid
WHO	World Health Organization
Milk	Unless otherwise stated, the word milk refers exclusively to cows' milk

1. INTRODUCTION

Vitamin A is considered one of the most important vitamins in both animal and human nutrition (Morrison, 1961). Dietary vitamin A is required for the maintenance of the normal processes of vision (Wald, 1960), growth (Olson, 1969), bone development (Roels, 1967), reproduction (Thompson, 1970) and synthesis and maintenance of the mucous secreting membranes (DeLuca and Wolf, 1969). Hypovitaminosis A is a major nutritional problem in many countries, particularly affecting post-weaning, preschool children.

Milk is one of the richest sources of vitamin A (Roels, 1967). However, the level of the vitamin in milk is under the influence of numerous factors. Some of the factors originate from the metabolism and dietary intakes of the dairy cow, while others originate from processing, marketing and handling of the milk after it has been obtained from the dairy producer.

The purpose of the present study was three-fold:

i) to determine the effect of stage of lactation, cow-to-cow variations, day-to-day variations, seasonal variations, and feeding and management of the dairy cow on the vitamin A and β-carotene content of milk.

ii) to determine the effect of processing (pasteurization, skimming and fortification), marketing and storage of milk prior to consumption, on the vitamin A potency of milk.

iii) to recommend possible measures which can minimize the conditions that cause unnecessary loss of vitamin A in milk and milk products.

2. REVIEW OF THE LITERATURE

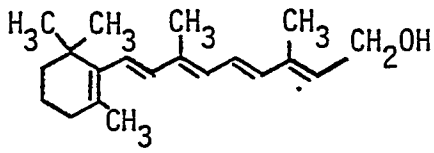
Vitamin A was independently discovered by McCollum and Davis and Osborne and Mendel in 1913-1915 (Roels and Lui, 1976). The vitamin, the first to be discovered, was initially identified as a growth promoting factor isolated from animal fats and oils. In 1930, Moore (1957) demonstrated that the carotenoids of plants were structurally related to vitamin A and were converted *in vivo* to the vitamin. Vitamin A and β-carotene were structurally formulated by Karrer and his coworkers in 1931 (Bäuernfeind and Cort, 1974). Despite its early identification, research workers did not begin to take a keen interest in vitamin A until the 1960's. Most of the available literature on vitamin A is concerned with its metabolism and biological roles but little work has been conducted on the factors affecting the availability of the vitamin in foods.

2.1 Physical and chemical properties of vitamin A

The generic term "vitamin A" refers to β-ionone derivatives which exhibit the biological activity of retinol. Vitamin A activity is demonstrated by retinol (the alcohol form), retinal (the aldehyde form), retinoic acid (the acidic form), and retinyl palmitate and retinyl acetate (the ester forms). Their formulae and structures are shown in Figure 1. Retinol is the most common form of vitamin A in nature. Retinol and its derivatives are exclusively of animal origin (Roels, 1967; Roels and Lui, 1976).

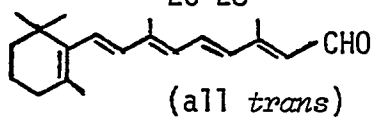
1. Retinol ($C_{20}H_{29}OH$)

empirical formula: 9,13-dimethyl-7-(1,1,5-trimethyl-6-cyclohexen-5-yl) -7,9,11,13-nonatetra en-15-ol



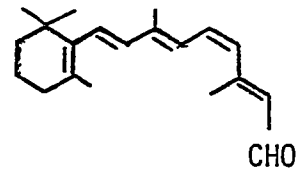
(all *trans* form)

2(a) Retinal ($C_{20}H_{28}O$)

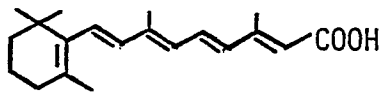


(all *trans*)

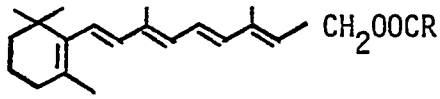
2(b) 11-*cis* retinal



3. Retinoic acid ($C_{20}H_{28}O_2$)



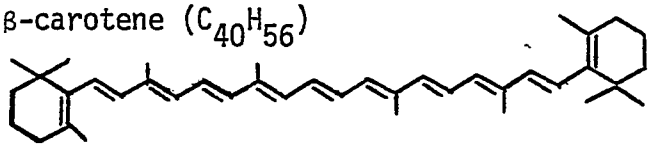
4. Retinyl ester



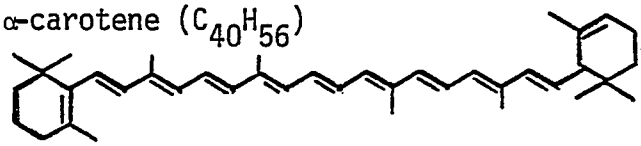
R = CH_3 retinyl acetate

R = $(CH_2)_{14}CH_3$ retinyl palmitate

5. β-carotene ($C_{40}H_{56}$)



6. α-carotene ($C_{40}H_{56}$)



7. γ-carotene ($C_{40}H_{56}$)

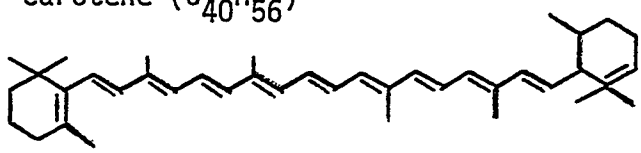


Figure 1 Structures of vitamin A and pro-vitamin A compounds (Olson, 1969).

Physically, vitamin A compounds melt at 63-64°C, boil at 120-125°C and are insoluble in water but soluble in organic solvents. The molecular weight of retinol is 286. Vitamin A compounds are stable to heat in the absence of oxygen but are unstable in the presence of oxygen (Bauernfeind and Cort, 1974; Roels and Lui, 1976). Vitamin A compounds are stable in alkaline conditions but unstable in acid media. The absorption maximum of retinol in ethanol is 324-325 nm (Strohecker and Henning, 1965). Ultraviolet light destroys vitamin A compounds (Satter et al., 1977). Vitamin A compounds can exist as either *trans* or *cis* isomers. Apart from 11-*cis*-retinal (Figure 1), which plays a role in the visual cycle, the *trans* isomers are the most biologically important isomers of vitamin A (Olson, 1969).

Vitamin A compounds are capable of undergoing a number of biological reactions. The most important are the oxidation-reduction reactions and esterification reactions which enable interconversion between the various forms of vitamin A. Retinol is oxidized *in vivo* to retinal by the retinol oxidase. The retinal can be either used in the visual cycle, reduced to retinol by retinal reductase or oxidized to retinoic acid. Retinoic acid cannot be biologically reduced to retinal (Ito et al., 1974; Thompson, 1975). Hence it can not function in metabolic pathways, such as vision and reproduction, which specifically require retinal or retinol. Retinol is interconverted to retinyl esters, *in vivo*, by a variety of retinyl esterases for storage and transport purposes.

2.2 Physical and chemical properties of β-carotene

About a hundred carotenoids are known to occur in higher plants, but only about ten of them exhibit any appreciable amount of vitamin A activity (Roels, 1967). Nutritionally, all-*trans*-β-carotene (Figure 1) is the most important provitamin carotenoid due to its high biological activity and its widespread occurrence in foods (Fernandez et al., 1976 a). Basically, β-carotene consists of two retinyl moieties joined by a double bond. β-carotene and its derivatives are essentially of plant origin (Roels, 1967), but they can be found in the tissues of those animals which consume and absorb β-carotene.

Physically, β-carotene has a melting point of 181°C. The molecular weight of β-carotene is 536. β-carotene is insoluble in water but soluble in organic solvents. Like retinol, β-carotene is readily oxidized in the presence of oxygen (Roels and Lui, 1976). The absorption maximum of β-carotene in chloroform is 451 nm (Bickoff, 1957; Strohecker and Henning, 1965). Ultraviolet light destroys β-carotene (Satter et al., 1977).

Other carotenoids with provitamin A activity include α- and γ-carotenes (Figure 1) and cryptoxanthine. Lycopene and xanthophyll have no provitamin A activity (Roles, 1967).

2.3 Biological activity of vitamin A and carotenoid compounds

The vitamin A and provitamin A activity of animal and plant tissue, respectively, is usually expressed in terms of International Units (hereinafter referred to as IU), in which one IU is defined as being equal to 0.344 μg all-*trans*-retinyl acetate, 0.30 μg all-*trans*-retinol (crystalline vitamin A alcohol) or 0.60 μg pure all-*trans*-β-carotene (Rodriguez and Irwin, 1972).

In 1967, the FAO/WHO expert committee recommended that retinol equivalents (RE) be introduced as the unit of measurement of vitamin A activity (FAO/WHO, 1967). One RE is equivalent to 1 µg all-*trans*-retinol or retinyl esters, 6 µg all-*trans*-β-carotene or 12 µg of any other carotenoid (FAO/WHO, 1967; Guthrie, 1975). The advantage of this system is that other provitamins having less biological activity than β-carotene are taken into account.

Retinol and retinyl esters have the highest biological activity among the vitamin A compounds. The biological activity of retinal and retinoic acid is 90% and 60% of retinol, respectively (Olson, 1969). The biological activity of carotenoids is judged by their ability to be converted to vitamin A *in vivo* (Roels, 1967). β-carotene has the highest biological activity. The biological activity of α- and γ-carotene is 50% that of β-carotene (Roels and Lui, 1976).

2.4 Vitamin A requirement of man

The establishment of a minimum requirement for vitamin A is complicated by the fact that the liver stores of vitamin A of an individual are not known and are influenced by age, sex, diet and physiological conditions. Methods used to determine the vitamin A status of an individual include growth rate tests, dark adaptation tests, depletion tests, serum vitamin A levels and liver vitamin A levels. Although liver reserves of vitamin A are the best indicators of the vitamin status of an individual (Underwood et al., 1970), this method cannot be used on a living subject.

The determination of serum vitamin A levels is the easiest and most commonly used method to estimate the vitamin A status of an individual (Rodriguez and Irwin, 1972; Srikantia, 1975). This method, however, is a

poor estimator as serum vitamin A levels do not correlate to liver stores. Serum vitamin A levels decrease only after liver stores are depleted (Rodriguez and Irwin, 1972; Underwood et al., 1970). On the basis of serum vitamin A levels, an individual is classified as being vitamin A deficient if serum levels are below 10 µg retinol per 100 ml of blood. Normal serum vitamin A levels lie within the range of 20-50 µg retinol per 100 ml of blood (Srikantia, 1975).

The RDA for vitamin A of an adult human is estimated at 5000 IU (1500 RE) and 4000 IU (1200 RE) per day for men and women, respectively (Rodriguez and Irwin, 1972; Guthrie, 1975). This level of vitamin A intake is sufficient to prevent the occurrence of deficiency symptoms and to ensure adequate liver storage. The increased requirement during pregnancy and lactation requires an additional consumption of 200 and 400 RE per day, respectively. Infants require 400 RE per day and children require 700 RE per day in order to meet daily needs and to build up liver reserves (Rodriguez and Irwin, 1972). Depletion of the liver vitamin A reserves in humans is estimated to take from 60 to 600 days (Srikantia, 1975).

2.5 Food sources of vitamin A

Provitamin A sources, particularly leafy vegetables and vegetable oils, provide the major proportion of the human requirements of vitamin A. Fruits, roots (except carrots) and cereal grains (except yellow corn) contain negligible amounts of carotenes (Bridge and Spratling, 1966; Roels, 1967).

Animal products provide a smaller proportion of the vitamin A require-

ments of man than plant products because the former are expensive. Meats, particularly liver, fish, poultry and eggs are good sources of vitamin A (Roels, 1967). Milk and milk products are rich sources of vitamin A and contribute about 25% of the dietary vitamin A requirements in North America (Hartman and Dryden, 1974), 20% in Great Britain and over 50% in New Zealand (McGillivray, 1960).

2.6 Vitamin A requirement of livestock

Vitamins A and E have been termed the "limiting vitamins" in livestock nutrition (Church, 1972; Cullison, 1975) because their requirements can only be met through dietary means. The rest of the vitamins can be synthesized either in the rumen (vitamins C, K and the B vitamins), in metabolic pathways (vitamin C) or in the skin (vitamin D).

Cattle require an intake of 80, 150 and 130 µg β-carotene per kg live weight per day for growth and maintenance, for pregnancy and for lactation, respectively (Anon., 1965; Greenhalgh, 1970). NRC (1971) recommends a daily vitamin A requirement of 43 IU and 77 IU per kg body weight for maintenance and lactation and for maintenance and pregnancy, respectively. The recommendations for sheep are 50 and 100 µg β-carotene per kg live weight per day for growth, maintenance and pregnancy and for lactation, respectively (Anon., 1965; Greenhalgh, 1970). Pregnancy in sheep appears not to require an increased intake of vitamin A. Pigs require daily intakes of 4 and 12 µg retinol per kg live weight for fattening and breeding, respectively (McGillivray, 1960). Moore (1967) recommended that the requirements of poultry be given in units per kg ration and recommended a daily intake of 660 and 2190 µg vitamin A per kg ration for growth and breeding, respectively.

2.7 Factors affecting vitamin A and β-carotene utilization

2.7.1 Protein

A prolonged low-protein diet eventually leads to vitamin A deficiency. Vitamin A deficiency symptoms were observed in 40 - 60% of the children hospitalized for protein-calorie-malnutrition (PCM) although their liver stores of vitamin A were within the normal range (Mahadevan et al., 1965; Srikantia, 1975; Thompson, 1975; Pereira and Begum, 1976). Upon feeding protein to these children, the vitamin A deficiency symptoms disappeared although vitamin A had not been fed. A possible explanation is that protein plays an important role in the absorption, synthesis from β-carotene, transport and storage of vitamin A.(Mahadevan et al., 1965; Roels and Mark, 1972; Stohecker and Anrich, 1973). Stohecker and Anrich (1973) reported higher rates of conversion of β-carotene to vitamin A and intestinal absorption of vitamin A on a 40% protein diet than on a 20% protein diet.

Dietary protein, however, exerts its greatest influence on vitamin A metabolism through the synthesis of retinol binding protein (hereafter referred to as RBP) which is essential for vitamin A transport in the blood (Goodman, 1970; 1974). Recently, Venkataswany et al. (1977) reported that protein-calorie-malnutrition (PCM) children showing signs of xeropthalmia had low serum levels of RBP. Dietary vitamin A did not elevate the RBP levels but dietary protein restored RBP levels to normal within one week. Protein deficiency results in inadequate amounts of RBP being synthesized. As a consequence, vitamin A cannot be adequately mobilized from the liver. Glover and Muhilal (1976) demonstrated that supplementing a pure rice diet with methionine and lysine significantly increased the level of RBP in the livers of weanling rats.

2.7.2 Lipids, vitamin E, thyroxine and zinc

The quantity and quality of dietary fat influence the metabolism of vitamin A and β-carotene in the digestive tract. Dietary fat enhances the conversion of β-carotene to vitamin A and also increases the absorption of retinol across the intestinal mucosal cells (Bridge and Spratling, 1966). Pereira and Begum (1976) reported that adding 18 gm of vegetable oil to the diets of young boys increased their serum vitamin A levels from 20 to 30 µg retinol/100 ml and reduced fecal losses of carotene and vitamin A from 90 to 55 percent.

Vitamin E is essential for the normal *in vivo* utilization of vitamin A (Bridge and Spratling, 1966; Bauernfeind and Cort, 1974). Vitamin E has a "sparing effect" on vitamin A requirements. Being an antioxidant, vitamin E protects vitamin A from oxidation in the intestinal tract and also enhances vitamin A absorption and storage in the liver (Hartman and Dryden, 1974).

Thyroxin stimulates intestinal conversion of β-carotene to vitamin A. Feeding β-carotene to thyroidectomized rats did not alleviate vitamin A deficiency symptoms (Moore, 1957). Thyroxin also enhances vitamin A absorption and storage in the liver (Church, 1972).

Zinc is known to be necessary for the maintenance of normal concentrations of vitamin A in the blood. Smith et al. (1973) postulated that zinc was needed for the synthesis of RBP which is needed for vitamin A transport in the blood. This was later confirmed by Brown et al. (1976) who demonstrated that zinc deficiency decreased the vitamin A level in the blood of rats and farm animals. Furthermore, feeding massive doses of vitamin A did not eleviate serum vitamin A levels but dietary zinc restored

the normal levels of serum vitamin A.

2.8 Metabolism of vitamin A and β-carotene

2.8.1 Enzymatic conversion of β-carotene into vitamin A

β-carotene is converted to vitamin A in the intestinal mucosa of almost all animal species by the enzyme β-carotene-15-15'-dioxygenase (Moore, 1957; Glover, 1960; Ganguly, 1969; Olson, 1969). The mechanism of conversion is essentially oxidative cleavage starting either at the terminal end (Glover, 1960; Lowe et al., 1956) or at the central bond (Olson, 1969). Only one molecule of retinal, which is subsequently reduced to retinol, results from the reaction.

The efficiency of conversion varies amongst species. The rat and the chick have the highest efficiency of conversion; 2 µg β-carotene are converted to 1 µg retinol (Aitken and Hankin, 1970; Fernandez et al., 1976 a). For man, 4 µg β-carotene are required to make 1 µg retinol (Campbell and Marshall, 1975). The efficiency of conversion, in ruminants, has been found to be low. Martin et al. (1969) demonstrated that, for ruminants, 1 mg of carotene from corn silage was equivalent to 0.24 mg all-*trans*-retinyl palmitate. This means that approximately 5 µg β-carotene are converted into 1 µg retinol. Many authorities (FAO, 1967; Greenhalgh, 1970; NRC, 1971; Church, 1972) are in agreement that 1 mg β-carotene is equivalent to 400 IU retinol for ruminants. The cat is an extreme, being incapable of converting β-carotene into vitamin A (Aitken and Hankin, 1970; Thompson, 1975).

The utilization of the β-carotene that is not converted into vitamin A is also species and strain specific. Man, cattle, horse, chicken and rabbit absorb

and circulate the unconverted carotene but goats, sheep, pigs, dogs and rats do not absorb the unconverted carotene (Church, 1972; Hartman and Dryden, 1974). Among cattle, Guernseys have the highest and Holsteins the lowest ability to absorb the unconverted carotene.

2.8.2. Digestion, absorption, storage and mobilization of vitamin A

All forms of ingested vitamin A, except retinoic acid, are eventually converted to retinol in the small intestine (Thompson, 1975). The retinol is actively absorbed by the intestinal mucosal cells. Within the mucosal cells, 75% of the absorbed retinol is re-esterified to form retinyl palmitate (Ganguly, 1969). Retinyl palmitate is incorporated into chylomicrons and transported, via the lymphatic system, to the systemic blood system. The vitamin A in excess of immediate utilization is stored in the liver, as retinyl palmitate. The liver contains 90% of the vitamin A reserves in the body (Ganguly, 1969; Olson, 1969). Retinoic acid enters the portal blood directly; not via the lymphatic system. Retinoic acid is not stored in the liver (DeLuca and Roberts, 1969).

Retinol is the transport form of vitamin A in the blood. Retinol has been found to circulate in the blood bound in a 1:1 ratio to a specific carrier protein called Retinol Binding Protein (RBP) (Goodman, 1970; 1974; Underwood, 1974). The RBP is, in turn, bound to prealbumin (PA). Interaction of retinol with RBP is of considerable physiological importance. The interaction serves to solubilize the otherwise insoluble retinol; protects the unstable retinol from chemical degradation and prevents retinol from exerting its surface activity in a non-specific way. Interaction of RBP with PA prevents the relatively smaller molecule of RBP from being lost through glomerular filtration (Goodman, 1970; 1974). Retinoic Acid Binding Protein (RABP) has recently been isolated from the cytosol and nuclei of

rat, mouse and chick embryo skin (Psnai, 1977). The function of cytosolic RABP is, clearly, that of specifically transporting retinoic acid. However, the role of RABP in the nucleus has not been elucidated.

Most of the vitamin A, in excess of immediate use and liver storage, is converted to retinoic acid in the liver. The terminal 14 and 15 carbons of retinoic acid are decarboxylated and lost as carbon dioxide (DeLuca and Roberts, 1969) while the intact ring is excreted in urine and feces (Olson, 1969). The rest of the retinoic acid molecule is conjugated with uridine diphosphoglucoronic acid to form retinyl-β-glucoronide which is excreted in the bile (Olson, 1969; Roels and Lui, 1976).

2.8.3 Mammary transfer of vitamin A and β-carotene from blood into milk

The major forms of vitamin A and vitamin A precursors in the blood, in the post-absorptive state, are retinol, retinyl palmitate and β-carotene. The healthy mammary gland has the capability of transferring retinyl palmitate, small quantities of retinol and β-carotene from the blood into the milk (Hartman and Dryden, 1974). The mammary gland can also esterify retinol to retinyl palmitate before transferring the ester into the milk. Under normal conditions, milk contains a greater proportion of retinyl palmitate and a smaller proportion of retinol and β-carotene than the blood.

2.9 Vitamin A: Biological roles and deficiency states

The physiological functions of vitamin A have been known for many years. Vitamin A is necessary for growth, reproduction, bone metabolism, normal vision and normal metabolism of the epithelial tissues (Thompson, 1975).

The main causes of vitamin A deficiency are inadequate intake or

impaired metabolism. The former is the more common. Vitamin A deficiency is a major nutritional health problem in many developing countries. The problem is severest among preschool children, amongst whom, vitamin A deficiency is the primary cause of preventable blindness (Srikantia, 1975). Vitamin A deficiency problems are aggravated by the poor nutritional status of the mother during pregnancy and lactation and by the poor quality foods to which the infant may be weaned. The child, therefore, does not get an opportunity to develop adequate liver reserves of the vitamin and is susceptible to deficiency diseases (Srikantia, 1975; Pereira and Begum, 1976).

The problem of vitamin A deficiency is not restricted to developing countries; it is world-wide. Nutritional surveys in Canada and the Ten-State Nutritional Survey in the United States revealed that vitamin A, iron, and calcium are the three essential nutrients most likely to be supplied in marginal amounts in the diets of these populations (Sauberlich et al., 1976). Results of a Canadian survey conducted by Hoppner et al. (1969) revealed that 15-34% of the autopsy subjects analyzed had reserves of less than 40 µg retinyl palmitate per gm liver (wet tissue). The results indicate marginal vitamin A intakes since adequate hepatic reserves of vitamin A are in the region of 40-100 µg retinyl palmitate per gm liver tissue. Subsequently, Underwood et al. (1970) determined the vitamin A content of the livers of subjects dying from unnatural causes in New York city. They also reported that 35% of the American subjects tested had less than 40 µg retinyl palmitate/gm liver (wet tissue). Symptoms of actual hypovitaminosis have been observed in some populations of Americans (Chopra and Kevany, 1970).

2.9.1 Role of vitamin A in the visual cycle

The biochemical reactions involved in the visual cycle were first identified by Wald (1943). The visual cycle, as it is known today, is shown in Figure 2. Rhodopsin, the photo-receptor pigment in the rods of the retina of most vertebrates, consists of the protein opsin covalently attached to 11-*cis*-retinal (Wald, 1960; Roels, 1967). Upon absorption of a quanta of light, the 11-*cis*-retinal in rhodopsin isomerises to all-*trans*-retinal via the intermediates, lumi-rhodopsin and meta-rhodopsin. This conformational change causes the detachment of opsin from all-*trans*-retinal (Wald, 1943; 1960; 1968; Roels, 1967). Rhodopsin must be regenerated quickly for the visual process to be continuous. The series of reactions involved in regeneration is shown in Figure 2.

The recovery time of rhodopsin after exposure to light has been found to be proportional to the amount of vitamin A in the blood (Roels and Lui, 1976). Hence, people with vitamin A deficiency are often unable to quickly adjust to dim light after being in bright light. This condition, known as night blindness, is the oldest and commonest vitamin A deficiency sign in the adult. The condition disappears upon consumption of high-vitamin A foods.

2.9.2 Role of vitamin A in the maintenance of the metabolism of epithelial cells

Vitamin A is needed for the maintenance of the normal functioning of epithelial tissues of mucous secreting systems such as the respiratory tract, reproductive tract, gastro-intestinal tract, the eye, tooth enamel and the skin (Wolf and Johnson, 1960). Vitamin A enhances incorporation of esterified sulfate into mucopolysaccharide (Roels, 1967; Srikantia,

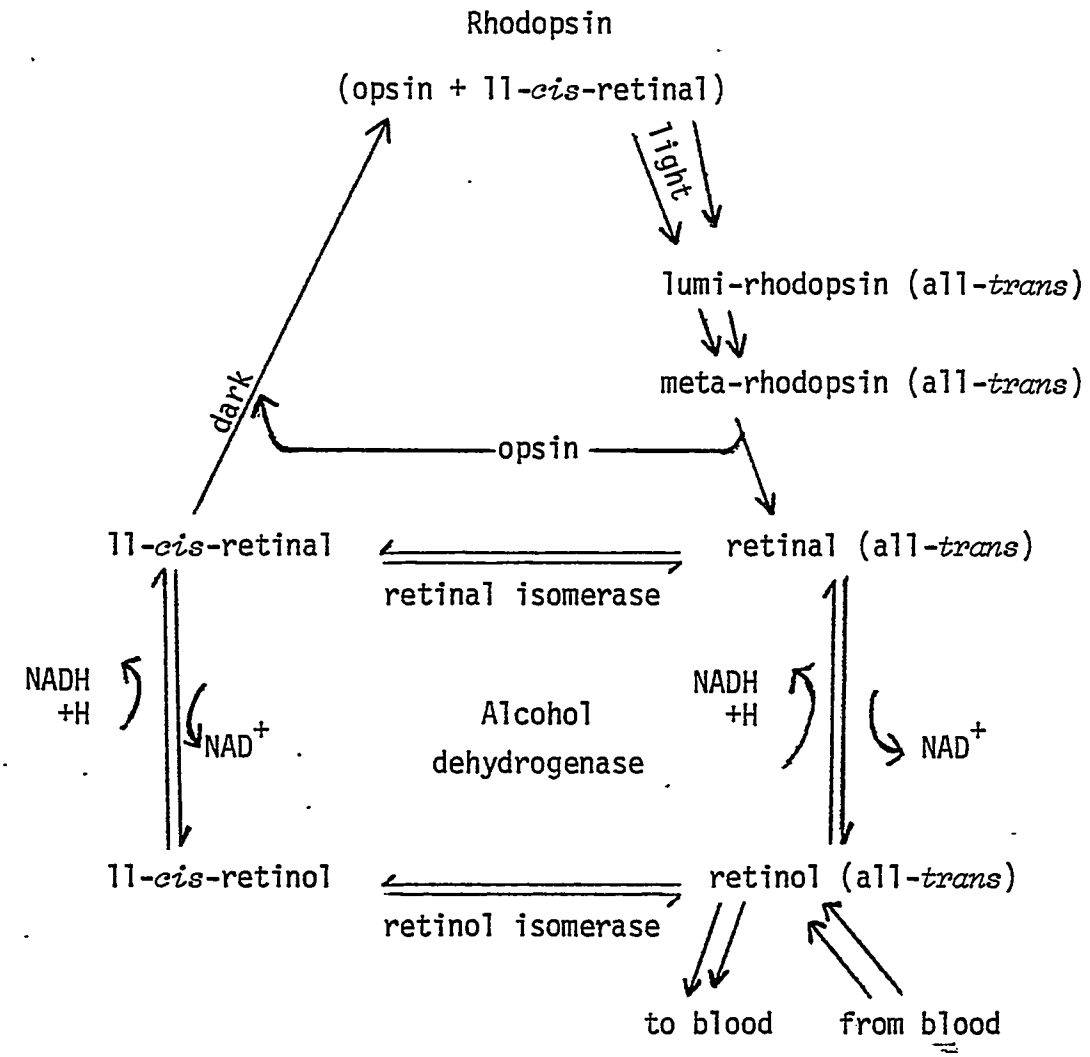


Figure 2. The role of vitamin A in the visual cycle (Wald, 1960; Roels, 1967).

1975). Mucopolysaccharide is a major component of mucous. In the presence of vitamin A, epithelial cells are capable of producing mucous, but produce keratin in the absence of the vitamin.

In the vitamin A deficiency state, the level of esterified sulfate, in mucous secreting tissues, is lowered, resulting in the inhibition of the synthesis of mucopolysaccharide (Roels, 1967). Srikantia (1975) reported that vitamin A-deficient children not only excreted low amounts of mucopolysaccharide, but the sulfated fraction also constituted only 30% of total mucopolysaccharides as compared to 70% in normal children. The process of keratinization is enhanced in vitamin A deficiency (DeLuca and Wolf, 1969). Keratinized cells peel off and pile up, creating a favourable environment for the growth of micro-organisms. These concepts will be used to explain the conditions prevailing in mucous secreting systems in the presence and absence of vitamin A.

2.9.2.1 The eye

Vitamin A is needed to maintain the normal functioning of the eye. In the absence of the vitamin, especially in children two to four years old, a condition known as xerophthalmia develops in the conjucativa area. This consists of two types of lesions: xerosis and Bitots spots (Srikantia, 1975). The former is characterized by the drying of the conjuctiva cells and the latter by foamy silver-grey spots on the bulba conjuctiva (Srikantia, 1975; Thompson, 1975). The situation is still reversible at this stage. However, when the cornea becomes involved, the situation becomes an emergency. In advanced stages of the deficiency, the cornea becomes hazy, dry and subsequently softens (keratomalacia). Blindness in one or both eyes is almost always the result (Srikantia, 1975). FAO (1974) estimated that

200,000 children in the world go blind, in this way, every year.

2.9.2.2 Respiratory tract, digestive tract, and the skin

Vitamin A was originally called "Anti-infective A" because it increases resistance to infections by maintaining the mucous lining of the respiratory tract. Respiratory infections may occur as a secondary effect of vitamin A deficiency. Vitamin A is needed for the normal process of intestinal cell proliferation (Zile et al., 1977). Its absence leads to the production of more gastric juices and less mucous; resulting in gastric ulceration and diarrhea. Vitamin A maintains differentiation of the epithelial cells of the skin. In the absence of vitamin A, the skin becomes dry and rough due to keratinization of the hair follicles. Such skin problems may not be due to vitamin A deficiency alone, but to a combination of a deficiency of vitamin A and essential fatty acids (Srikantia, 1975; Thompson, 1975).

2.9.3 Role of vitamin A in reproduction

Vitamin A is necessary for the maintenance of normal reproduction in animals. In the male, vitamin A is needed to maintain spermatogenesis and testicular development. In the female, vitamin A maintains a normal level of circulating sex hormones which are needed for reproductive purposes. The vitamin is also needed by birds for normal egg production and hatchability. Glover et al. (1976) reported that the plasma concentration of holo-RBP increased immediately prior to the breeding season of sheep; emphasizing the need for increased mobilization of vitamin A for reproductive purposes.

Vitamin A deficiency, in the male, results in atrophy of the accessory sex organs and the degeneration and loss of the germinal epithelia of the

testes (Thompson, 1970). Spermatogenesis is thus impaired. In the female, disruptions of the oestrous cycle and infections in the reproductive tract occur. Thompson (1970) reported fetal resorption in the second week of pregnancy in rats maintained on vitamin A deficient diets. In livestock, male animals have lowered sperm count and viability. Female animals have lowered conception rates, congenital malformations, stillbirths and birth of weak, disease-prone calves (Swanson et al., 1968). In the domestic fowl, vitamin A deficiency results in decreased egg production and decreased hatchability (Thompson, 1970; Thompson, 1975).

The effects of vitamin A deficiency on the reproductive capacity of the female can be explained by keratinization and endocrine disturbances. The pathway for conversion of cholesterol to sex hormones is blocked in vitamin A deficiency, resulting in a decrease in the level of circulating steroid hormones (Guthrie, 1975). In the male, the effects of vitamin A deficiency are due to a disruption in the normal processes of cell differentiation (Thompson, 1970).

2.9.4 Vitamin A and bone metabolism

Vitamin A is needed during bone remodelling for the release of proteolytic enzymes from lysosomes which are needed to break down the intracellular matrix of cartilage. An optimal concentration of vitamin A is required in order to balance the rate of bone formation with the rate of bone breakdown. Vitamin A is also needed for the synthesis of chondroitin sulfate, a major component of the extracellular matrix of cartilage (Church, 1972).

In vitamin A deficiency states, bone overgrowth and formation of soft bones has been observed in experimental animals (Roels, 1967). Vitamin A

exerts its effect on bone metabolism through sulfate metabolism (Roels, 1967) but not through calcium metabolism (Zile et al., 1973). Vitamin A may also affect bone metabolism via enzymatic activity and collagen metabolism. Zile et al. (1973) found decreased concentrations of alkaline phosphatase, an enzyme concerned with bone formation and resorption, in bone and plasma of vitamin A-deficient rats. They also found increased hydroxyproline levels in the plasma of vitamin A-deficient rats, an indicator of collagen breakdown.

The effect of vitamin A deficiency on bone metabolism is very pronounced in livestock. Abnormal bone structure leads to blindness in calves due to constriction of the optic nerve (Morrison, 1961; Thompson, 1970). Increased cerebral spinal fluid pressure (CSFP) of vitamin A-deficient sheep, pigs or calves (Thompson, 1975) is due to abnormal remodelling of skull bones. Poor bone formation causes pressure on the central nervous system (CNS) which leads to nervous disorders, incoordination and paralysis, particularly in pigs (Thompson, 1975).

2.9.5 Vitamin A and growth

Vitamin A is needed for the maintenance of normal growth rates. The growth promoting activity of retinoic acid equals that of retinol (Ito et al., 1974). The growth failure observed in vitamin A deficiency could be due to decreased food intake resulting from keratinization of taste buds (Olson, 1969; Guthrie, 1975; Thompson, 1975). Vitamin A deficiency could also depress growth by decreasing the circulating level of growth hormones (Olson, 1969).

2.9.6 Role of vitamin A in the maintenance of cellular membrane stability

Retinol and retinoic acid, in collaboration with vitamin E, have been shown to regulate the stability of cellular membranes (Roels and Lui, 1976). Optimal vitamin A concentrations in the blood ensure optimum cellular membrane stability by maintaining the normal level of hydrolytic enzymes in the cell. Roels et al. (1969) observed that large- or deficient-doses of vitamin A resulted in the release of large amounts of hydrolytic enzymes from lysosomes; causing labilization of hepatic cellular membranes *in vivo* and *in vitro*.

Sudhakaran and Kurup (1974) demonstrated that a dietary intake of vitamin A within the range of 100 - 2,000 IU/rat/day ensures stability of cell membranes but, outside this range, membrane stability decreases. Apart from the action of vitamin A on cell membrane stability via hydrolytic enzymes, vitamin A may act directly on the lysosomal membranes by disrupting membrane structure (Roels et al., 1969). Sudhakaran and Kurup (1974) found a decrease in the phospholipid content of liver cell membranes in vitamin A-deficient rats. This suggests that vitamin A participates in lipid-protein interactions in cellular membranes.

2.9.7 Role of vitamin A in differentiation of cancerous cells

Recent evidence indicates that intakes of large doses of retinoids tend to reverse the development of epithelial cancer in experimental animals. Retinyl methyl ether has been found to be very effective in reversing mammary cancer (Grubbs et al., 1977). Shamberger (1971) reported that retinyl acetate and retinol reduced the incidence of skin tumors by 76%. β-carotene did not reduce the incidence of the skin tumors. The actual mechanism by which vitamin A influences differentiation of cancerous cells is not clearly understood. Enhancement of lysosomal activity by vitamin A

has been suggested. According to Shamberger (1971), large doses of vitamin A compounds enhance lysosomal activity resulting in the release of cyclophosphamide which exerts chemopeutic inhibition of tumor growth.

2.10 Interaction of vitamin A with carbohydrate, lipid and protein metabolism

Vitamin A plays an important role in glycoprotein synthesis by acting as a carrier of monosaccharide units to acceptor proteins (Roels, 1967). Vitamin A also increases synthesis of glycogen from glycerol, acetate and lactate by increasing the activity of glycogen synthetase and decreasing the activity of glycogen phosphorylase (Wolf and Johnson, 1960). Roels (1967) reported a decrease in glycogen synthesis in vitamin A-deficient rats. Vitamin A appears to ensure adequate carbohydrate reserves in the body. In addition, Church (1972) reported that the rate of hepatic gluconeogenesis in vitamin A-deficient rats was reduced.

Vitamin A metabolism has been found to be linked with that of coenzyme Q and the biosynthesis of cholesterol (Roels, 1967). Lowe et al. (1956) observed an increase in the levels of hepatic coenzyme Q (ubiquinone) and a decrease in the biosynthesis of cholesterol in vitamin-A deficient rats. The incorporation of mevalonic acid into ubiquinone and squalene is enhanced in vitamin A deficiency but that into cholesterol is depressed.

Protein plays a major role in vitamin A metabolism (Section 2.7.1) but the role of the vitamin in protein metabolism is still controversial. Moore (1960) stated that vitamin A had no specific influence on protein metabolism. On the contrary, Roels et al. (1972) reported that vitamin A influences the *in vivo* synthesis of serum proteins; the *in vitro* synthesis of muscle proteins and the *in vitro* synthesis of glycoproteins in the intestinal

mucosa of rats and cellular membranes. The mechanism whereby vitamin A influences protein metabolism is not clearly known (Roels et al., 1972).

2.11. Prevention of vitamin A deficiency

2.11.1 Fortification of foods with vitamin A

Swaminathan et al. (1970) carried out a field survey in which children, in the danger zone of one to five years,were administered a massive annual dose of 300,000 IU retinol per child to reverse vitamin A deficiency symptoms and to build up adequate liver reserves. This, however, tended to result in toxicity problems. Fortification of foods with vitamin A is a more appropriate physiological approach to the eradication of deficiency than annual or semi-annual administration of massive doses of vitamin A. Fortification involves no change in the dietary habits of the target group hence readily available foods can be used. The major drawback of fortification of food with vitamin A is loss of activity of the vitamin due to oxidation by light, high temperatures and long storage periods (Bauernfeind and Cort, 1974).

Over fifty foods have been fortified with vitamin A. These include salad oils, margarine, cereals, peanut butter, ice cream, flour, cakes, rice, soft drinks and baby foods (Bauernfeind and Cort, 1974). Fortification of salt, sugar (Srikantia, 1975) and tea (Pereira and Begum, 1976) has been suggested, as these are commonly consumed food items.

2.11.1.1 Milk and milk products

The efficiency of utilization of vitamin A and β-carotene provided by milk and milk products is superior to that provided by any other food (Thompson, 1968). Milk is a good vehicle for fortification of vitamin A

as it is consumed by infants and children who need more vitamin A for growth. When butterfat is removed from milk, the fat-soluble vitamins are also removed. These nutrients need to be returned to skim milk (non-fat milk), partly skimmed milk (2% butterfat) and dry skim milk powder (DMP).

Fortification of milk with vitamin A is accomplished by using either a water-dispersible, stabilized beadlet carrier of synthetic retinyl palmitate or a retinyl-palmitate emulsified concentrate (Hartman and Dryden, 1974). In some countries, for example, Canada, consumers prefer a yellow tinge in butter and β-carotene is used to fortify winter butter. Several problems encountered in fortification of dairy products are taste, flavour, stability and uniform distribution (Bauernfeind and Cort, 1974). A hay-like off-flavour has been identified in vitamin A-fortified milk (Jenness and Patton, 1959; Lampert, 1975). Vitamin A itself is the origin of the off-flavor (Jenness and Patton, 1959). Use of a high quality vitamin A concentrate has been observed to lessen the development of the hay-like off-flavor in milk.

2.11.2 Non-fortified food sources of vitamin A

In areas where animal products are expensive and fortification programs are not feasible, the people are encouraged to eat large amounts of green vegetables (Pereira and Begum, 1976). Rao and Rao (1970) compared the absorption of carotenoids from leafy vegetables, root vegetables and fruits with crystalline β-carotene, in four volunteer adults. The absorption of β-carotene from green foods was reported to be fair to good, ranging from 30-60% efficiency. Sweeney and Marsh (1970) reported that liver stores of rats fed carrots were equivalent to those of animals fed all-*trans*-β-carotene

dissolved in cotton seed oil.

2.12 Toxic effects of excess vitamin A intake

Vitamin A has, like vitamin D, the property of being toxic when consumed in excessive amounts. Single doses of 12,000 IU/kg body weight for adults and single doses of 300,000 IU/day for infants have been found to be toxic. A prolonged daily dose of 400 IU/kg body weight can also be toxic to adults (Thompson, 1970; Rodriguez and Irwin, 1972). Seventy-five percent of the acute cases of hypervitaminosis occur in infants, due to the actions of overzealous mothers.

Fell (1970) demonstrated that excess vitamin A caused the release of acid hydrolases, which caused a breakdown of intercellular mucoprotein, resulting in bone resportion in the rat and chick. Studies with both experimental animals and humans showed that RBP transporting capacity was exceeded in times of excess vitamin A intake (Smith and Goodman, 1976). The excess vitamin A, circulating unbound in the blood, may then exert its surface active effects non-specifically.

Symptoms of acute hypervitaminosis A in humans include loss of hair, vomiting, bone and joint aches and severe headaches (Neuman et al., 1954, Roels and Lui, 1976). Excess vitamin A may lead to the disruption of vitamin K metabolism; resulting in prolonged clotting time and severe hemorrhage (Neuman et al., 1954). In livestock, particularly calves, Thompson (1975) reported decreased Cerebral Spinal Fluid Pressure on ingestion of 3,200 µg retinol/kg live weight/day.

2.13 Methods of determining vitamin A and β-carotene contents of biological materials

2.13.1 Determination of vitamin A in biological materials

Commonly used methods of vitamin A analysis include biological (Johnson, 1948), colorimetric (Strohecker and Henning, 1965), spectrophotometric (Underwood, 1974; Parrish, 1977) and fluorescent methods (Thompson et al., 1972; Senyk et al., 1975). Apart from the biological methods, all methods involve an initial extraction of vitamin A from the unsaponifiable fraction of the fat; followed by a determination of the concentration of the vitamin utilizing its various properties. Only the colorimetric method will be discussed here.

2.13.1.1 Colorimetric methods: Antimony trichloride method vs trifluoroacetic acid method

Vitamin A, due to the polyene structure of its side chain, reacts with a number of highly acidic compounds to yield blue-colored products having an absorption maxima at 620 nm (Underwood, 1974). The antimony trichloride method, developed by Carr and Price, is the oldest method of determining vitamin A in biological materials. The method gives the closest correlation between the depth of the blue color and the vitamin A concentration in the test material. However, it is gradually being replaced by the trifluoroacetic acid (hereafter referred to as TFA) method for the following reasons: i) antimony trichloride is very sensitive to moisture and traces of moisture cause it to become turbid; ii) the blue color formed by the oxidation of vitamin A with antimony trichloride is very unstable and fades rapidly, making it difficult to obtain an accurate absorption measurement; and

iii) the antimony trichloride is extremely corrosive. TFA retains the sensitivity and specificity of antimony trichloride, but it is not sensitive to moisture and the blue color, formed by the oxidation of vitamin A with TFA, is more stable (Neeld and Pearson, 1963; Dugan et al., 1964; Bradly and Hornbeck, 1973).

2.13.2 Determination of β-carotene in biological materials

The quantitative determinations of β-carotene consists of three essential steps: i) hot or cold extraction of the carotenoid pigments from the plant or animal tissue using a suitable fat solvent (Bickoff, 1957; Knowles et al., 1972); ii) separation of β-carotene from the other plant carotenoids such as xanthrophylls and chlorophylls using chromatographic techniques (Ass. Vit. Chem., 1966) and iii) measurement of the carotene content of the purified extract at 450 nm (Bickoff, 1957).

2.14 The nutritive value of milk and milk products

The nutritive value of milk depends on its composition (Figure 3). Therefore, factors which affect the components of milk will inevitably affect the nutritional quality of the milk.

2.14.1 Vitamins in milk and milk products

Milk is a fair source of both the fat-soluble and the water-soluble-vitamins (Figure 3). The levels of vitamins A, E and D in milk and milk products are influenced by the level of these vitamins or their provitamins in the feed of the dairy cattle (Hartman and Dryden, 1974). The levels of vitamins C and K and the B-vitamins, however, are not influenced by dietary factors (Cullison, 1975).

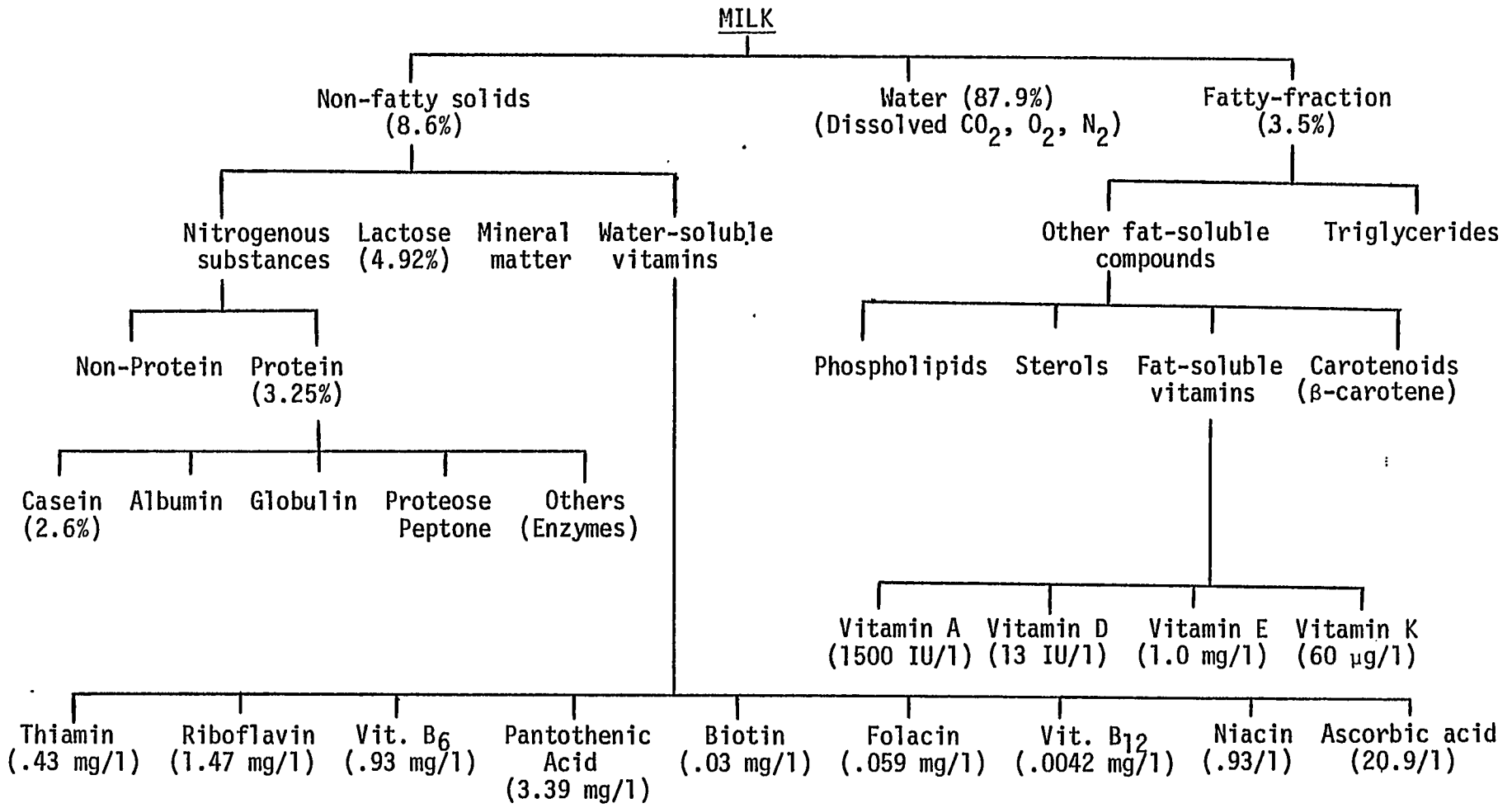


Figure 3. Constituents of milk. Partly adapted from Ling et al. (1961); Hartman and Dryden (1974).

2.14.2 Vitamin A and β-carotene contents of milk and milk products

The term vitamin A "activity" or "potency" of milk is used to refer to the vitamin A plus β-carotene content of milk (Smith, 1967; Schmidt, 1971). Milk is a good source of vitamin A. According to Roels (1967), milk is the best source of preformed vitamin A. The vitamin A activity of fluid milk, butter and cheese (cheddar) is about 150 IU per 100 ml, 2,920 IU per 100 gm and 1,170 IU per 100 gm, respectively (Hartman and Dryden, 1974). About 98% of the preformed vitamin A content of milk is in the form of retinyl esters while carotenoids, particularly β-carotene, make up 11 to 55% of the total vitamin A potency (Kirtchgesner et al., 1967; Hartman and Dryden, 1974). Practically all of the vitamin A and β-carotene in milk is associated with the unsaponifiable portion of the fat (Jennes and Patton, 1959; Lampert, 1975). However, there are indications that the vitamin A and carotenoids in milk are present in both the fat-globule membrane and in the protein complex (Hartman and Dryden, 1974; Campbell and Marshall, 1975). Consequently, the quantity of vitamin A in milk is only roughly proportional to the fat content of milk.

2.15 Factors affecting the vitamin A and β-carotene contents of milk and milk products

The vitamin A activity of milk and milk products is subject to the influence of many factors. The beneficial effects of vitamin A can only be optimized if these factors are known and precautions are taken to minimize those conditions which cause unnecessary losses.

2.15.1 Effect of processing and marketing factors

No appreciable loss in the vitamin A activity of milk occurs when milk is pasteurized, evaporated, sterilized or dried (Wanner, 1973; Hartman and Dryden, 1974; Owen and McIntire, 1975). Pasteurization alone causes little or no loss to the vitamin but when followed by evaporation, a 20% loss was observed (Thompson, 1968; Hartman and Dryden, 1974). Ultraviolet irradiation of milk to convert 7-dehydrocholesterol (provitamin D_3) to cholecalciferol (vitamin D_3) was reported to have no apparent effect on the vitamin A activity of the milk (Hartman and Dryden, 1974; Satter and deMan, 1975). On the contrary, Thompson (1968) reported that this process causes isomerization of all-*trans*-vitamin A to *neo*-vitamin A which has decreased biological activity.

Vitamin A in fortified milk is less stable than that naturally present in milk (Thompson and Erdody, 1974). The vitamin A that is added to skim milk is destroyed at a higher rate than that added to whole milk (Campbell and Marshall, 1975) because the former lacks the protective effects of fat. These facts emphasize the need for proper handling of fortified skim milk. Storage of milk at low temperatures, dim light and in an oxygen-free environment results in negligable loss of the vitamin A activity. High temperatures, however, increase the rate of loss of vitamin A and β-carotene. Hartman and Dryden (1974) reported that the vitamin A activity of sterilized milk was not affected by a six month storage period at 4 to 20°C but lost 48% of the vitamin at 38°C after only six weeks of storage. Storage of butter at -18°C for one year resulted in no appreciable loss of vitamin A (Thompson, 1968).

Fluorescent light, used for display purposes in food stores, can result

in photolysis of vitamins, particularly riboflavin and vitamin C (Hedrick and Glass, 1975) and vitamin A and β-carotene (Satter and deMan, 1975). Wavelength can determine the extent of photo-degradation of vitamin A. Satter et al. (1977) demonstrated that maximal loss of vitamin A and β-carotene occurred at wavelengths below 415 and 465 nm, respectively. They recommended proper packaging of milk to block all wavelengths below 465 nm. β-carotene or its light-induced decomposition products can cause an overall reduction in incident light energy and hence "protect" vitamin A from photo-degradation. Satter et al. (1977) demonstrated that β-carotene, at concentrations of 2.5 to 7.5 μg/ml, significantly protected vitamin A from photo-degradation. This finding has obvious applicability in the dairy industry.

2.15.2 Effect of the dairy cow

2.15.2.1 Breed variations

Different breeds of cattle show marked differences in their ability to utilize dietary β-carotene. These differences are reflected in the carotene:vitamin A ratio of their milk. Under similar feeding conditions, Guernseys and Jerseys produce milk containing more carotene than the Holstein and Ayrshire breeds. However, the latter breeds yield more preformed vitamin A (Thompson, 1968; Hartman and Dryden, 1974; Lampert, 1975). This is because Guernseys and Jerseys convert a smaller proportion of dietary carotene into vitamin A than the Holsteins and Ayrshires. Milk from Guernsey cows have more vitamin A activity per ml because of a higher butterfat content but milk from Holsteins have more total vitamin A activity because Holsteins yield a larger volume of milk. The vitamin A content of milk from the

different breeds of cattle are not significantly different when the vitamin A activity is expressed on the basis of the fat content of the milk (Greenhalgh, 1970; Lampert, 1975).

2.15.2.2 Effect of stage of lactation

The vitamin A activity of colostrum is 10 to 20 times the activity of normal milk (McGillivray, 1960). A slight increase in the vitamin A content of milk at the end of the lactation period was reported by Rakes (1965) and Thompson (1968). However, McGillivray (1960), Ling et al. (1961), Smith (1967), Hartman and Dryden (1974) reported that the stage of lactation had no influence on the vitamin A activity of the milk.

2.15.2.3 Effects of diseases of the udder (mastitis)

Mastitis impairs the ability of the mammary gland to convert vitamin A alcohol to vitamin A ester. The resulting milk, therefore, has been found to contain more vitamin A alcohol than vitamin A ester (Owen, 1965; Schalm et al., 1971). The total vitamin A content of the milk, however, remains unchanged. The total carotenoid content of milk from cows suffering from mastitis is higher than that of milk obtained from healthy cows (Schalm et al., 1971). Mastitis has been found to cause increased permeability between blood and milk and, as there are more carotenoids in blood than in milk, the carotenoid level in milk increases.

2.15.3 Effect of type of feed

Feed and feeding practices cause the greatest variations in the vitamin A and β-carotene contents of milk.

2.15.3.1 Pasture

Among natural forages, pasture provides the most plentiful dietary source of carotenes for the cow. During the months of pasture feeding, β-carotene contributes more than 50% of the total vitamin A potency of milk (Hartman and Dryden, 1974). However, not all the dietary β-carotene from pasture is converted into vitamin A. β-carotene is best utilized at quite low levels of intake and the efficiency of conversion to vitamin A decreases with increasing dietary levels (Kirchgessner et al., 1967; Thompson, 1968). Experimental evidence shows that a daily intake of 2.5 g β-carotene per cow is all that is required to produce milk with maximal vitamin A potency (McGillivray, 1960; Greenhalgh, 1970; Hartman and Dryden, 1974). At daily intakes greater than 2.5 g β-carotene/cow, a transient rise in the vitamin A content of the resulting milk was reported (Hartman and Dryden, 1974).

The stage of maturity and the botanical composition of a pasture influence its β-carotene content. McDowall and McGillivray (1963) reported that the β-carotene content of pasture decreased with increasing maturity of the pasture. They also reported that a predominance of clover species can significantly reduce the β-carotene content of a pasture.

2.15.3.2 Conserved forages (silage and hay)

Conserved forages contain much less β-carotene than green forages. Extensive oxidative breakdown and loss of β-carotene occurs when forage plants are harvested for purposes of conservation (Kirchgessner et al., 1967). The breakdown is enhanced by sunlight, long storage periods and high temperatures (Morrison, 1961). The greatest loss (90%) occurs when hay is sun cured in the field (Kirchgessner et al., 1967). Artificially dried hay loses only about 10 to 20% of its original β-carotene content

(Smith, 1967). Hay fed in pellets results in less vitamin A activity in milk than chopped hay (Rakes and Potts, 1965; Smith, 1967).

Much less β-carotene is lost when a crop is made into silage than when it is cured into hay (Morrison, 1961; Kirchgessner et al., 1967; Thompson, 1968; Schmidt, 1971). Kirchgessner (1967) reported a level of 3.9 µg vitamin A per gm butterfat in butter obtained from milk of cows fed hay as compared to 9.47 µg per gm butterfat in butter of cows fed silage. The type of crop ensiled and its stage of maturity affect the β-carotene content of the silage. Legume silage contains more carotene than grass silage (Smith, 1967). Corn silage is only a fair source of β-carotene because, in addition to its high content of cryptoxanthine (Bridge and Spratling, 1966), the corn plant is not ensiloed until it is relatively mature.

2.15.3.3 Seasonal variations

The seasonal variations observed in the vitamin A and β-carotene contents of milk reflect the response to different levels of β-carotene in the feed at different times of the year. Summer milk has been found to contain 1.5 to 15 times more vitamin A activity than winter milk (Schmidt, 1971; Hartman and Dryden, 1974). Searles and Armstrong (1970) reported a total potency for Alberta (Canada) butter of 22.60 IU/g fat in winter and 30.50 IU/g fat in summer. They found that milk has maximal vitamin A potency in the spring when fresh pasture became available and minimal values in late winter. Dornbush et al. (1940) reported that the Wisconsin (USA) market milks had highest vitamin A activity in October and lowest activity in

March. In winter, cattle not only receive less carotene but the little they get contains a higher percentage of inactive carotenoids. McDowall and McGillivray (1963), Thompson (1968), Hartman and Dryden (1974) reported that 40-75% of the carotenoids in winter milk was inactive as compared to 5-25% in summer milk.

The stage of maturity of a forage crop significantly influences the seasonal variation in the vitamin A and β-carotene content of milk. McDowall and McGillivray (1963) observed a higher vitamin A activity in the milk of cows fed immature rye grass than in the milk of cows fed mature rye grass. The mature grass had a high content of lipids, primarily leaf cuticle wax, which may decrease conversion of β-carotene to vitamin A. Furthermore, winter milk in New Zealand has been found to contain more vitamin A activity than summer milk as the pasture in winter is young and green while that in summer is mature and dry (McGillivray, 1960; McDowall and McGillivray, 1963; Thompson, 1968).

2.15.3.4 Dietary β-carotene vs dietary vitamin A: their effect on the vitamin A potency of milk

Cattle can meet virtually all of their vitamin A requirements by ingesting β-carotene in good quality forages. However, dietary β-carotene is insufficient to provide an adequate vitamin A activity in the milk for human nutritional requirements. The efficiency of conversion of β-carotene to vitamin A by ruminants is low, due to a number of possible factors. The peculiar physiology of the ruminant digestive tract (Fernandez et al., 1976 b) may create unfavourable conditions for β-carotene metabolism. In addition, *in vivo* experiments using cattle and sheep and *in vitro* experiments, using rumen liquor, have shown that 10% of the dietary β-carotene (Fernandez

et al., 1976 a) and 40% of the dietary carotene (Church, 1972) are degraded and lost in the pre- and lower-intestinal tract, respectively. Furthermore, a high percentage of dietary β-carotene may be lost through faeces. Fernandez et al. (1976 b) obtained fecal recoveries of dietary β-carotene ranging from 92 to 98% and speculated that this was due to inefficient absorption of β-carotene.

The poor utilization of β-carotene by ruminants, outlined above, coupled with the fact that β-carotene cannot be available all year round emphasize the need for an alternative source of vitamin A for cattle.

The vitamin A activity of milk can be increased more efficiently by feeding preformed vitamin A than by feeding β-carotene. A 10 to 20-fold increase in the vitamin A activity of milk has been observed when cows were fed preformed vitamin A (Smith, 1967) as compared to a 1.5 to 15-fold increase when cows were fed β-carotene (Hartman and Dryden, 1974). The method of administration of vitamin A to the animal (oral or intravenous injection) has been observed to not have any significant effect on the vitamin A content of milk (Hartman et al., 1976). However, the form of vitamin A given and the media in which it is given are important determinants of the vitamin A recovery in the milk. Injected retinol appeared in milk faster than injected retinyl palmitate (Tomlinson et al., 1970) but retinyl palmitate is better for oral administration because it is more stable than retinol. Vitamin A absorption is better from an emulsion than from an oil-based solution (Kirchgessner et al., 1967; Hartman and Dryden, 1974).

The only major problem encountered with feeding preformed vitamin A is its instability in the intestinal tract. About 60% of the dietary

vitamin A is degraded and lost in the intestinal tract of ruminants (Church, 1972). This loss can be reduced by simultaneous feeding of antioxidants, such as tocopherol, with the vitamin A.

3. MATERIALS AND METHODS

3.1 Materials

Milk samples were obtained from the Dairy Producers' Co-operative, Saskatoon, Saskatchewan. Milk, blood and fecal samples were also obtained from the University dairy herd through the Department of Animal Science, University of Saskatchewan. Wheat silage, alfalfa hay and concentrate feeds were supplied by the Department of Animal Science.

Pure all-*trans*-vitamin A alcohol and pure all-*trans*-β-carotene standards were purchased from North America Scientific Co. Acetone (CH_3COCH_3), acetic anhydride ($(CH_3CO)_2O$), and alcohol (anhydrous, 95% alcohol by volume) were purchased from Baker Chemical Co., Phillipsburg. Chloroform ($CH{\cdot}Cl_3$), ethyl ether, kerosene and potassium hydroxide were from Baker Chemical Co. Petroleum ether (boiling range 30-40°C), pyrogallol ($1,2,3(CHO)_3C_6H_3$), trifluoroacetic acid (F_3CCOOH) and xylene ($C_6H_4(CH_3)_2$) were from Baker Chemical Co. Infusorial earth (white, calcinated) and propylene glycol ($CH_3COCHCH_2OH$) were purchased from Fisher Scientific Co., USA. Magnesium oxide (MgO) and hexane ($(CH_3(CH_2)_4CH_3)$, boiling range 67-70°C), were from British Drug House (BDH) Laboratory reagents. All reagents used were of analytical reagent grade.

3.2 Analytical methods

The colorimetric measurements for vitamin A and β-carotene were made with a Unican SP 1750 ultraviolet spectrophotometer. The butterfat

concentration of the milk samples were determined using a Milko-tester, MK III f.3140 with a Digitizer f.3121, at the Dairy Producers' Co-operative, Saskatoon. All procedures for the determination of vitamin A and β-carotene in test materials were carried out in dim light.

3.2.1 Calibration of vitamin A standard curve

A vitamin A standard solution was prepared by dissolving a known amount, approximately 0.050 gm, of vitamin A alcohol in 200 ml of ethyl ether in a pre-weighed volumetric flask (foil covered). A working standard was prepared by making a 1:50 dilution of the standard solution in ethyl ether. The concentration of the working standard was calculated using the following formula:

$$[\text{vitamin A}] = \frac{\text{vitamin A (mg)}}{\text{volume of standard (200 ml)}} \times \text{dilution factor} = \text{mg/ml}$$

Several aliquots of the working standard (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 ml) were taken up and made up to 4.0 ml, in triplicate, in ethyl ether. The samples were brought to dryness under a stream of nitrogen and the absorbance determined by the colorimetric method described in section 3.2.3, Step III. The standard curve was obtained by plotting absorbance of pure vitamin A vs concentration of vitamin A. The concentration of vitamin A in the assay solutions of all subsequent experiments was calculated by reference to the standard curve.

3.2.2 Calibration of the standard curve for β-carotene

The procedures used for the preparation of the β-carotene standard and the determination of the standard curve were identical to those used

for vitamin A with the exception that chloroform was used as the solvent and the working standard solution aliquots ranged from 0.125 to 1.0 ml. The absorbance of β-carotene in the standard solutions was measured at 450 nm against a chloroform blank.

3.2.3 Determination of vitamin A in milk

The method used for the determination of vitamin A in milk was a modification of the methods of Neeld and Pearson (1963) and Dugan et al. (1964) as adopted by the Saskatchewan Feed Testing Laboratory, University of Saskatchewan.

Extraneous fat-soluble substances present in milk interfere with the determination of vitamin A and necessitate purification prior to analysis. These substances were removed by saponification followed by extraction; leaving vitamin A in the unsaponifiable fraction of the fat portion of the sample.

Step I Saponification

A 50 ml aliquot of a thoroughly mixed 1 litre sample of milk was placed in a 250 ml erlenmeyer flask. After adding 75 ml of distilled water, the sample was flushed with a stream of nitrogen, covered with aluminum foil and placed in a hot-water bath (60°C) for 30 minutes. In the order listed, 10 ml of propylene glycol, 25 ml of 1% pyrogallol in ethanol (w/v) and 10 ml of 50% KOH (w/v) were added to the sample. The sample was then mixed, flushed with nitrogen and placed in a hot-water bath (70°C) for 1.5 hours. The saponified mixture was allowed to cool overnight.

Step II Extraction of vitamin A from the unsaponifiable fraction

The cooled, saponified mixture was washed with a 250 ml separatory funnel with ethyl ether and successively extracted with three 50 ml portions of ethyl ether. The pooled ether extracts were washed three times with 50 ml portions of distilled water and brought to 200 ml with ethyl ether.

Step III Color development

Three 10 ml aliquots of the ether extract were flushed with nitrogen and brought to dryness in a warm water-bath (40°C). Three drops of acetic anhydride (added to remove traces of moisture), 0.3 ml of chloroform (added to solubilize the vitamin) and 3.0 ml of 2:1 chloroform:trifluoroacetic acid mixture were added to the vitamin A residue and immediately mixed for 10 seconds. The absorbance was measured at 620 nm, against a reagent blank, exactly 30 seconds after the addition of the chloroform:TFA mixture.

Vitamin A is oxidized by trifluoroacetic acid (TFA) in chloroform; forming a transient blue-colored complex. The colorimetric reaction obeys Beer's Law between 10^{-6} to 10^{-5}M vitamin A. The amount of vitamin A in milk, expressed as IU per 100 ml or IU per gm butter fat, was obtained from the calculation formula in Appendix 1A.

3.2.4 Determination of the β-carotene content of milk

Procedures used for the saponification and extraction of β-carotene from milkfat were identical to those used for vitamin A. Three 10 ml aliquots of the ether extract were flushed with nitrogen and brought to dryness in a warm water-bath (40°C). Three ml of petroleum ether were added to the β-carotene residue, mixed for 5 seconds and the absorbance

measurements taken at 450 nm against a petroleum ether blank. The β-carotene content of the milk, expressed as IU per 100 ml or IU per gm butter fat, was obtained from the calculation formula in Appendix 1B.

3.2.5 Determination of vitamin A in concentrate feeds fortified with vitamin A

The determination of vitamin A in concentrate feeds fortified with vitamin A was essentially carried out by the method described in Section 3.2.3. In order to obtain a representative sample, a large random sample (about 100 gm) was ground, thoroughly mixed and a 5 gm sample taken and used for the vitamin A determination. To avoid plugging of the separatory funnels, the extraction of vitamin A from feeds was carried out on a filtrate of the digested feeds. The amount of vitamin A in concentrate feeds fortified with vitamin A expressed as IU per kg feed, was obtained from the calculation formula in Appendix 1C.

3.2.6 Determination of vitamin A and β-carotene in blood

The vitamin A and β-carotene content of blood samples was determined by a modification of the trifluoroacetic acid method described by Neeld and Pearson (1963).

Blood samples were drawn from the jugular vein of the experimental animals. Serum was prepared by centrifuging the blood samples at 10,000 x g for 30 minutes. Duplicate 2 ml aliquots of the serum were transferred to glass stoppered test-tubes. Two ml of 1N KOH in 95% ethanol (added to break up the vitamin A-protein complex) was added; followed by 3 ml of petroleum ether. The serum and reagents were stirred vigorougly for 2 minutes to ensure complete extraction of the vitamin A and β-carotene.

The samples were then centrifuged at 2,500 x g for 10 minutes. The absorbance of β-carotene was determined directly on the petroleum ether extract at 450 nm, against a petroleum ether blank. For vitamin A determination, two ml of the petroleum ether extract was brought to dryness in a warm water-bath (40°C). The residue was then taken up in 0.1 ml chloroform, 0.1 ml acetic anhydride and 1.0 ml of 2:1 chloroform:TFA mixture. After 10 seconds of mixing, the absorbance of the sample was determined at 620 nm, exactly 30 seconds after addition of the chloroform: TFA mixture. The vitamin A and β-carotene content of the blood samples, expressed as IU/100 ml, was calculated using the formulae in Appendix 1D.

3.2.7 Determination of vitamin A and β-carotene in fecal samples

The method for the determination of vitamin A and β-carotene in fecal samples was essentially that proposed by Embree et al. (1957) except that TFA was used in the determination of vitamin A.

A 50 gm sample was obtained from a fresh, well mixed rectal sample and placed in a 500 ml erlenmeyer flask. Exactly 200 ml of 6% alcoholic KOH was added to the flask. The contents were mixed and the flask placed in a hot water-bath (60°C) for 6 to 7 hours. The digested mixture was allowed to cool overnight, filtered and the filtrate brought to 100 ml with alcoholic KOH. Ten ml aliquots were transferred to 250 ml separatory funnels and extracted with 10 ml of petroleum ether or 10 ml of a 1:1 mixture of kerosene-xylene (v/v). The extract was brought to 50 ml with petroleum ether. Three 5 ml aliquots were brought to dryness in a warm water-bath (40°C). The absorbance of vitamin A in the sample was determined using the method described in Section 3.2.3, Step III. The absorbance of β-carotene in the petroleum ether extract was determined at 450 nm, against a petroleum

ether blank. The vitamin A and β-carotene content of the fecal sample expressed as IU/100 gm fresh fecal material, was obtained using the calculation formula in Appendix 1E.

3.2.8 Determination of β-carotene in silages and hays

The method for the determination of β-carotene in silages and hays was adopted from that proposed by AOAC (1975) with the exception that hot extraction was used instead of blending.

Step I Extraction

The β-carotene content of leaves is higher than that of stems. In order to obtain a representative sample, a 100 gm primary sample was randomly collected, freeze dried (silage) or oven dried (hay), then ground and thoroughly mixed. A 5 gm sample was placed in a reflux flask; 30 ml of 30% acetone-hexane mixture (v/v) added and the flask placed in a warm water-bath (40°C) for 1 hour. The refluxed mixture was allowed to cool overnight, filtered and the filtrate brought to 100 ml with hexane.

Step II Separation of pigments

β-carotene must be chromatographically separated from the biologically inactive carotenoids (chlorophylls and xanthrophylls) as these interfere with the β-carotene determination. A 400 x 20 mm chromatography column was half-filled with a 9% acetone-hexane mixture (v/v). A 1:1 mixture of infusorial earth and magnesium oxide (w/w) was then added with slight mixing until the column was well packed. The extracted sample was placed on the column and eluted, under suction, with 9% acetone-hexane (v/v). The eluate was brought to 200 ml with the eluent. The optical density of the β-carotene in the sample was measured at 450 nm, against a blank of 9%

acetone-hexane solution. The β-carotene content of the silage and hay samples, expressed as mg β-carotene per kg forage material, was obtained using the formula in Appendix 1F.

3.3 Wavelength effect on the photo-degradation of vitamin A and the "protective" effects of β-carotene

The influence of incident light on the stability of vitamin A in ethyl ether was studied in order to determine the exact wavelength or wavelength range at which maximal photo-degradation of vitamin A occurred. A working standard solution of vitamin A was prepared as described in section 3.2.1. Two ml aliquots of the solution were placed in quartz cuvettes and exposed to light of specific wavelengths (range 260-600 nm) for either 10 or 20 minutes. The concentration of vitamin A in the standard solution was determined by absorption at 325 nm, prior to and after exposure to light of a specified wavelength. The temperature of the sample was maintained at 15°C throughout the exposure period.

The "protective" effect of β-carotene on the light-induced decomposition of vitamin A in ethyl ether was also studied. β-carotene was added to the vitamin A solution to yield either a 1:1 or a 1:2 ratio of vitamin A:β-carotene mixture. The above procedures were repeated, exposing the vitamin A—β-carotene mixture at that wavelength range at which maximal photo-degradation of vitamin A occurred.

3.4 Some factors affecting the vitamin A and β-carotene content of milk

In all the experiments carried out to determine the factors affecting the vitamin A and β-carotene content of milk, the vitamin A and β-carotene assays were carried out following the procedures described in sections

3.2.3 and 3.2.4. Butterfat percentages of the milk samples were also determined in order to express the vitamin A activity of the milk as IU per gm B.F.

3.4.1 Effect of pasteurization on the vitamin A and β-carotene contents of 3.25% B.F. milk

In order to determine the effect of pasteurization on the vitamin A activity of the milk, milk samples with butterfat content standardized to about 3.25% were analysed monthly for a period of one year, June 1977 to June 1978. Two 1 litre samples of milk, one obtained from the homogenizer prior to and another obtained after pasteurization were used for vitamin A and β-carotene assays.

The results were expressed as IU vitamin A or β-carotene per 100 ml of milk. One-way analysis of variance was employed to determine whether the differences in the vitamin A content of unpasteurized and pasteurized milk were significant.

3.4.2 Effect of fortification on the vitamin A concentration in 2% B.F. milk and skim milk

The vitamin A content of unfortified and fortified 2% B.F. milk and fortified skim milk was analyzed monthly for the period of one year, June 1977 to June 1978. The vitamin A assay on the 2% B.F. milk (unfortified) was intended to determine the effect of partial fat removal on the vitamin A content of the milk. The 2% B.F. milk (fortified) and skim milk (fortified) were analyzed to determine the efficiency of fortification. The results were expressed as IU vitamin A/100 ml of milk. One way analysis of variance was employed to determine significant differences in the vitamin

A content of the milk.

3.4.3 Effect of storage on the vitamin A activity of milk

The deterioration rate of vitamin A with storage, the effect of storage temperatures and the importance of milk fat in "protecting" vitamin A from oxidation were studied. Four milk samples, skim milk (0.05% B.F.), partially skimmed milk (2% B.F.), homogenized milk (3.25% B.F.) and whole milk (3.88% B.F.) were stored at 5°C for a period of 21 days. Duplicate samples were stored at -15°C for the same period. Vitamin A assays and pH recordings were carried out every seven days. The results were expressed as IU vitamin A per 100 ml of milk. The deterioration rate of vitamin A potency was expressed as percent loss of the original potency.

3.4.4 Effect of time of milking on the vitamin A activity of the milk

In order to determine whether the time of milking (morning or evening) had any effect on the vitamin A content of the milk, milk samples were collected from four Holstein cows, in the same lactation period, for a period of three days. Morning and afternoon samples were obtained and analyzed separately. The vitamin A content of the milk was expressed as IU/100 ml, IU/gm B.F. and IU/milking. One way analysis of variance was employed to determine whether the effect of time of milking was significant.

3.4.5 Comparison of the vitamin A activity of milk from the fluid and industrial bulk tanks

Fluid milk producers have to meet a higher level of cleanliness than industrial milk producers. Milk obtained from the fluid and industrial milk storage silos located at the Dairy Producers Co-operative Plant in Saskatoon, was analyzed monthly for a period of one year to determine whether the quality of the milk affects its vitamin A content. The results were expressed as IU

vitamin A or β-carotene per 100 ml. One way analysis of variance was used to determine significant differences in the vitamin A content of the milk.

3.4.6 Seasonal variation in the vitamin A and β-carotene contents of Saskatoon market milk

"It is only by the examination of large bulk samples that the true seasonal effect on the vitamin A activity of milk, can be found", Smith (1967).

Seasonal variations in the vitamin A and β-carotene content of milk were determined by analysing the vitamin A and β-carotene content of monthly milk samples, for the period of June 1977 to June 1978. Five bulk milk samples were obtained from 5 representative areas around Saskatoon, Saskatchewan. Samples were taken from dairy farms in Rosthern, Warman, Waldheim, Viscount-Guernsey and Asquith regions situated within a 50-mile radius in north-eastern, northern, north-western, south-eastern and western sections about the city of Saskatoon, respectively. From these bulk samples, a smaller sample (about 1 litre) was obtained for analytical purposes. The vitamin A and β-carotene content of the milk was expressed as IU/100 ml and IU/gm B.F. Total vitamin A potency (vitamin A plus β-carotene) was also calculated. Analysis of variance was employed to determine the extent of seasonal variations on the vitamin A potency of the milk. Significant differences between seasons were determined by the Student-Newman-Keuls' test according to Rohlf and Sokal (1969).

The amount of precipitation (rainfall and snowfall) for each of the experimental months, was also recorded in order that the state of pastures could be assessed.

As a cross-check on the effect of season on the vitamin A activity of

milk, a milk sample from the University herd was analysed monthly for the same period. As the University of Saskatchewan dairy herd is kept indoors all the year, the milk should, therefore, not reflect the effects of pasture and pasture foraging that would be expected to be observed in samples from the commercial dairy farms.

3.4.7 Effect of increasing the level of vitamin A in the feed on the vitamin A activity of milk of cows in two stages of lactation

The vitamin A levels in the feed of dairy cows in two stages of lactation were increased in order to determine the following:

i) The rate at which the vitamin A level in the milk rises upon supplementation and the rate of decrease upon removal of the vitamin A supplement.

ii) The effect of stage of lactation on the vitamin A content of milk.

iii) The extent of day-to-day and cow-to-cow variations in the vitamin A activity of the milk.

Ten Holstein dairy cows, five in the early stages of lactation and five in the late stages of lactation were randomly selected and used in this experiment. The experimental period was 26 days. During the first ten days, the cows received an intake of 8,200 IU vitamin A/kg feed. In the next ten days, the vitamin A level of the feed was increased to 87,600 IU vitamin A/kg feed. In the last six days of the experiment, the cows received 8,200 IU vitamin A/kg feed. Milk samples were collected every other day; morning and afternoon milk samples were pooled and samples analysed for vitamin A and β-carotene contents.

The vitamin A and β-carotene content of the milk was expressed as IU/100 ml, IU/gm B.F. and IU/day. Analysis of variance was employed to determine whether increased levels of vitamin A in the feed, stage of

lactation, cow-to-cow variations and daily variations had any effect on the vitamin A activity of the milk. Significant differences between treatments were determined by the Student-Newman-Keuls' test according to Rohlf and Sokal (1969).

3.4.8 Effect of supplementation of hay and silage with two levels of vitamin A on the vitamin A activity of milk

A 4 x 4 Latin square experiment was designed to determine and compare the rate of turnover of the β-carotene from hay and silage into vitamin A activity of milk from cows fed these forages. The experiment was also intended to determine whether recovery of dietary vitamin A and β-carotene in the milk was affected by the level of vitamin A in the feed.

3.4.8.1 Experimental periods

The experiment consisted of four periods, each of 28 days. An inclusive 4-day standardization period was interposed at the beginning of each period. Period I lasted from January 1st, 1978 to January 31st; period II: February 1st to February 28th; period III: March 1st to March 28th; and period IV: March 29th to April 25th.

3.4.8.2 Experimental animals

Four registered Holstein-Friesian cows, weighing between 550 and 650 kg and having had between one and four lactations were obtained from the University of Saskatchewan dairy herd. The cows (No. 83, 101, 115 and 174) were well over their lactation peaks. They were each yielding a daily volume of milk greater than 30 kg with about 3.0% B.F. The cows were housed individually in box stalls in a well insulated barn.

3.4.8.3 Experimental feeds

Two forages, hay and silage were used. The hay was a good quality,

first cut brome alfalfa hay with 88% DM. Proximate analysis, expressed on a dry matter basis, showed that the hay contained 15.40% crude protein (CP), 0.55% calcium, 0.23% phosphorous and 58 mg β-carotene per kg of forage material. A 55% TDN in dry matter was assumed.

The silage was a Neepawa wheat (mid dough) of good quality with 42% DM. Proximate analysis, expressed on a dry matter basis, showed that the silage contained 14.30% CP, 0.23% calcium, 0.20% phosphorous and 30 mg β-carotene per kg of forage material. A 67% TDN in DM was assumed.

The carotene content of the forages was determined at the beginning of each experimental period, following the procedures described in Section 3.2.8.

Two levels of vitamin A supplementation were used; either a low level of 250,000 IU/cow/day or a high level of 600,000 IU/cow/day. Vitamin A supplementation was achieved by adding a known amount of vitamin A to concentrate feeds at the time of ration preparation. The composition of the concentrate feeds used is shown in Table 1. The vitamin A content of the concentrate feeds (both the fortified and non-fortified feeds) was determined at the beginning of each experimental period following the procedures described in Section 3.2.5.

The experimental animals received about 10 kg DM/cow/day of either hay or silage. Concentrate levels in the diet were adjusted to meet the required level of vitamin A supplementation and NRC (1971) requirements of total digestible nutrients (TDN) for maintenance and milk production. The vitamin A supplemented concentrate feeds were pre-weighed and offered twice daily together with the unsupplemented concentrate and silage or hay. Water was given *ad libitum*. Feed intake of the experimental animals was

Table 1

Chemical composition of the dairy concentrate feed

Ingredient	Amount (lb)	TDN	CP	CA	P
Wheat	100	80	13.5	.05	.30
Oats	100	68	10	.12	.36
Barley	506	73	10	.05	.30
Soya Bean Meal	138	75	45	.20	.60
Rapeseed Meal	60	73	35	.60	1.00
Urea	4	--	280	---	---
Wheat & Bran	10	58	15.7	.10	1.20
Molasses	30	60	7	.10	.02
Aicel	18	--	--	15.50	20.50
Limestone	16	--	--	37.00	---
Blue salt	8	--	--	---	---
Min.-Vit. Mix	10	73	10.0	.05	.30
	1000				

Mineral-Vitamin Mix

Vitamin A (500,000 IU/gm)	91.0 g
Vitamin D_3 (200,000 IU/gm)	2.5 g
Vitamin E (500 IU/gm)	10.0 g
MnO	15.0 g
ZnO	12.5 g
CuO	5.0 g

This will yield a vitamin A concentration of about 100,000 IU/kg concentrated feed.

$$\left(\frac{91 \times 500{,}000 \times 2.2045}{1000} = 100{,}305 \text{ IU/kg}\right)$$

In the mineral-vitamin mix for the concentrate with no vitamin A, the vitamin A component was eliminated

recorded together with the feed weigh-back.

The Latin square design of the experiment (Table 2) was constructed in a manner to ensure that a given cow would be transferred from one level vitamin A on hay or silage to another rather than, for example, shifting from hay to silage and then back to hay. Constant changes in the diet of the cow disturbs their metabolism and may introduce more variables in the experiment.

3.4.8.4 Sample collection and analysis

All samples were kept at 5°C, for not more than 48 hours, before they were analysed. Milk samples were collected on the last three days of each experimental period. Morning and afternoon milk samples were collected at 4:30 a.m. and 3:30 p.m. respectively, pooled and analysed for vitamin A and β-carotene. Blood samples were drawn from the jugular vein of the experimental animals, on the last day of each experimental period. The blood samples were then analysed for vitamin A and β-carotene following the procedures described in Section 3.2.6. Rectal fecal "grab" samples were collected on the last 5 days of each experimental period, composited and analysed for vitamin A and β-carotene using the methods outlined in Section 3.2.7.

Lignin contents of the feed and fecal samples were measured to determine digestibility of the feeds. These analyses were carried out by the Feed Analysis Laboratory in the Department of Animal Science, using the Lignin method (Forage Fibre Analysis, 1970).

3.4.8.5 Statistical analysis

Analysis of variance was carried out for the Latin square design experiment to determine the effect of the experimental feeds on the

Table 2

The layout for the Latin square feeding experiment

	Cow Numbers			
Period	115	101	83	74
I	S_2	H_2	H_1	S_1
II	S_1	H_1	H_2	S_2
III	H_2	S_2	S_1	H_1
IV	H_1	S_1	S_2	H_2

S_1 Silage - low vitamin A level (250,000 IU/cow/day)

S_2 Silage - high vitamin A level (600,000 IU/cow/day)

H_1 Hay - low vitamin A level (250,000 IU/cow/day)

H_2 Hay - high vitamin A level (600,000 IU/cow/day)

vitamin A and β-carotene content of milk. Significant differences between treatments were determined by the Student-Newman-Keuls' test according to Rohlf and Sokal (1969).

4. RESULTS

4.1 Preliminary studies

A number of preliminary experiments were conducted to establish procedures and experimental conditions used in the analytical methods.

4.1.1 Length of the pause period between addition of TFA-chloroform mixture to the vitamin A residue and the reading of the absorbance

Vitamin A reacts with TFA in chloroform to form a transient blue-colored complex. Maximal color intensity occurs within seconds of the addition of the TFA-chloroform mixture, making the timing of the absorption measurement critical. Dugan et al. (1964), Bradly and Hornbeck (1973), and Hashmi (1973) recommended that the absorbance be taken 5 to 10 seconds after addition of the TFA-chloroform mixture to the vitamin A residue. However, this time period was found to be too short in which to conveniently take the reading. In order to select a suitable pause period, absorbance of the reaction products at 620 nm was measured at equally spaced time intervals (Figure 4). Thirty seconds was found to be the most convenience pause period, in accordance with the observations of Neeld and Pearson (1963).

4.1.2 The ratio of TFA:Chloroform mixture

Maximal absorption is affected by the proportion of TFA in the TFA: chloroform reaction mixture. Dugan et al. (1964) and Hashmi (1973) obtained good color yields using a 1:1 ratio of TFA:chloroform mixture. The findings of the present study are in agreement with those of Neeld and

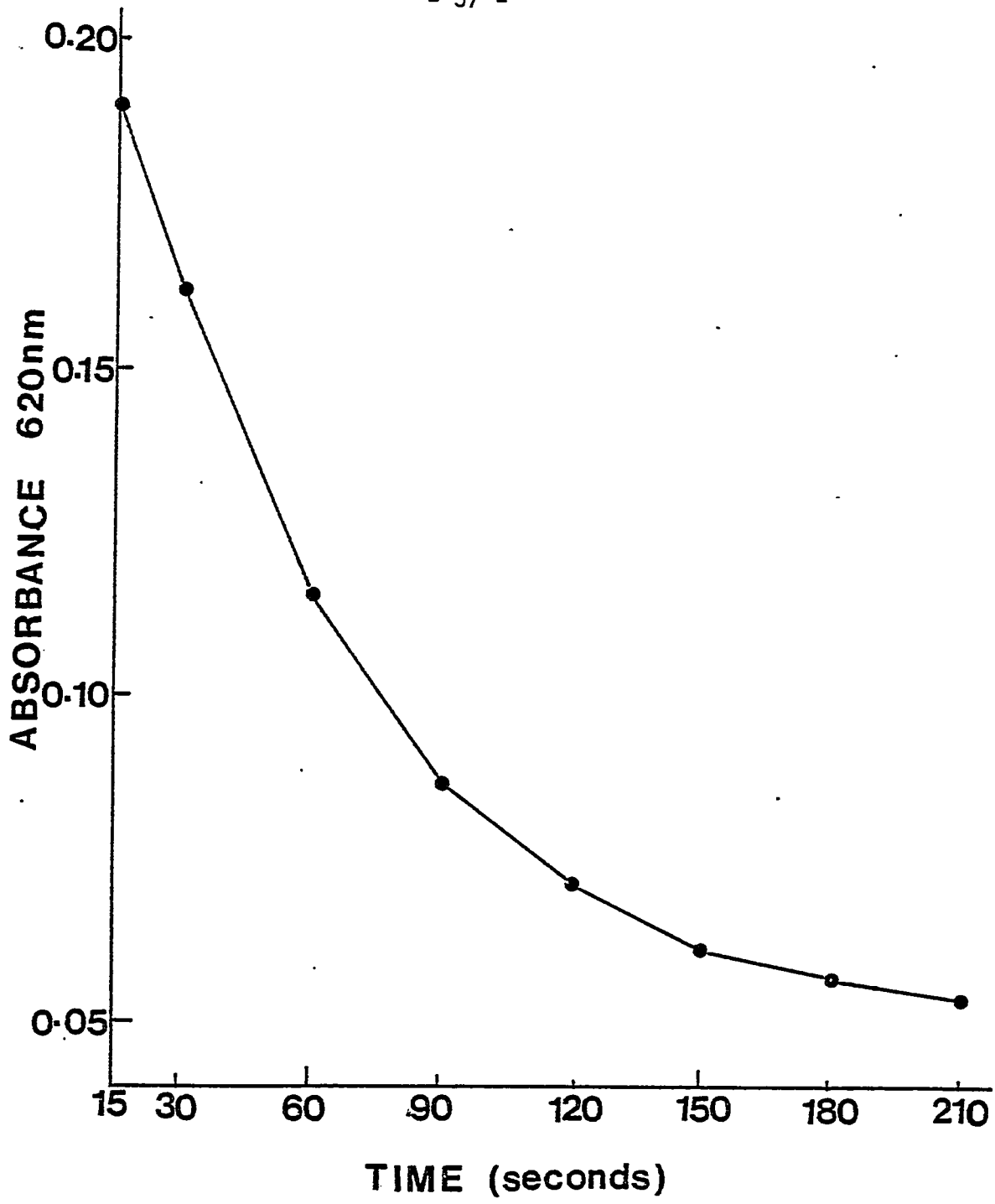


Figure 4. Time dependence of TFA:vitamin A absorbance at 620 nm.

Absorbance readings of aliquots (1.0 ml) of 1.6×10^{-3} mg vitamin A/ml in 3.0 ml of a 1:2 TFA:chloroform mixture, were measured at 620 nm. Time intervals of readings were at 15, 30, 60, 90, 120, 150, 180 and 210 seconds following initiation of reaction. Each point is a mean of 3 samples.

Pearson (1963) that a 1:2 ratio of TFA:chloroform mixture gives the best color yields. The ratio of 1:2 TFA:chloroform was, therefore, used throughout this study.

4.1.3 Absorption spectra of vitamin A, β-carotene, vitamin A–β-carotene mixture and TFA-vitamin A species

The absorption spectra of vitamin A, β-carotene solutions and a vitamin A-β-carotene mixture were determined in order to obtain absorption maxima for vitamin A and β-carotene. The absorption maxima are the wavelengths at which the light absorption is directly proportional to the concentration of the test material in the sample. The absorption maxima of vitamin A and β-carotene (Figure 5 and Figure 6) were found to be 325 nm and 450 nm, respectively, in agreement with the observations of Bikoff (1957), Strohecker and Henning (1965) and Hashmi (1973). The absorption spectra of a vitamin A-β-carotene mixture (Figure 7) showed no absorption interference between the two compounds. The absorption maxima for the TFA-vitamin A species was found to range between 616 and 620 nm in accordance with the observations of Dugan et al. (1964) and Neeld and Pearson (1963). The wavelength of 620 nm was used in all vitamin A assays.

4.2 Standard curves for vitamin A and β-carotene

Standard curves for vitamin A and β-carotene were plotted in order to standardize the analytical methods and to increase the degree of reproducibility. Linear regression analysis was employed and the resulting standard curves for vitamin A and β-carotene are shown in Figure 8 and Figure 9, respectively. Calculations of the concentration of vitamin A and β-carotene in all subsequent assays were made by reference to these standard curves.

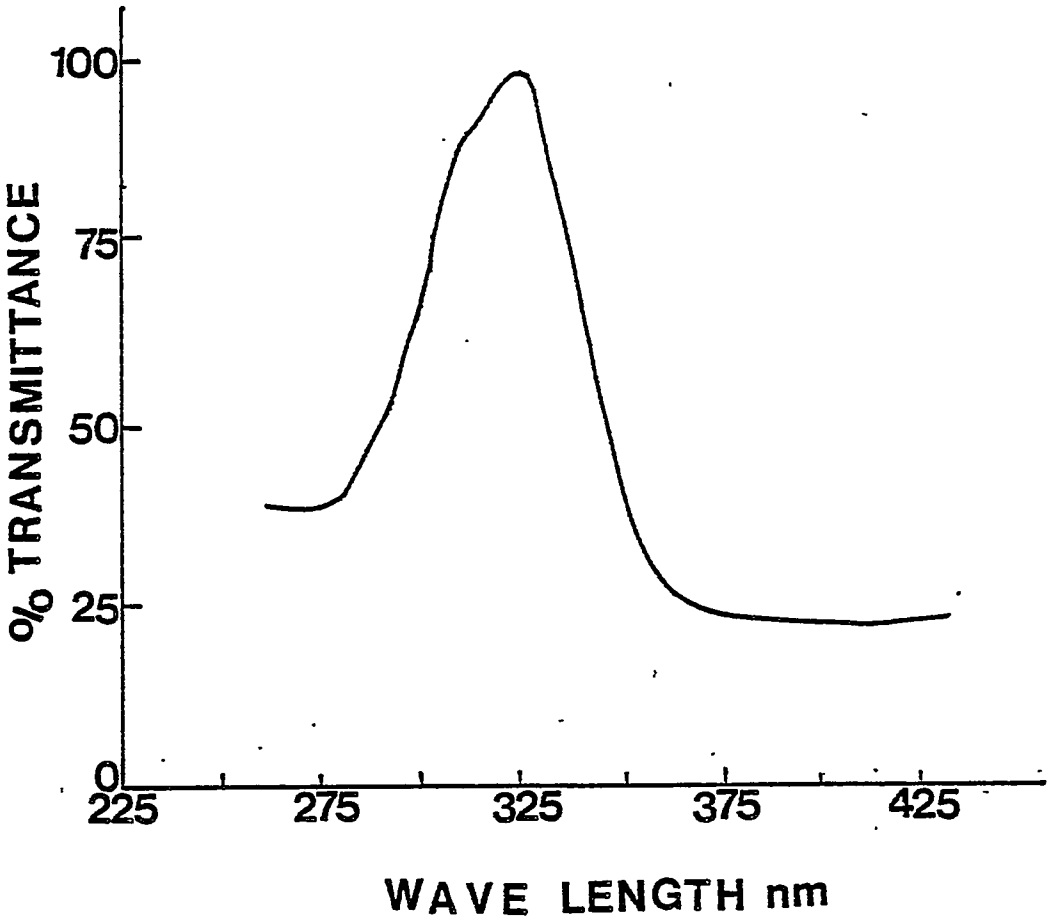


Figure 5. Absorption spectrum of vitamin A in ethyl ether.

Absorption spectrum (250 to 425 nm) of a 2.0 ml sample of 1.6×10^{-3} mg vitamin A/ml in a 1-cm light path.

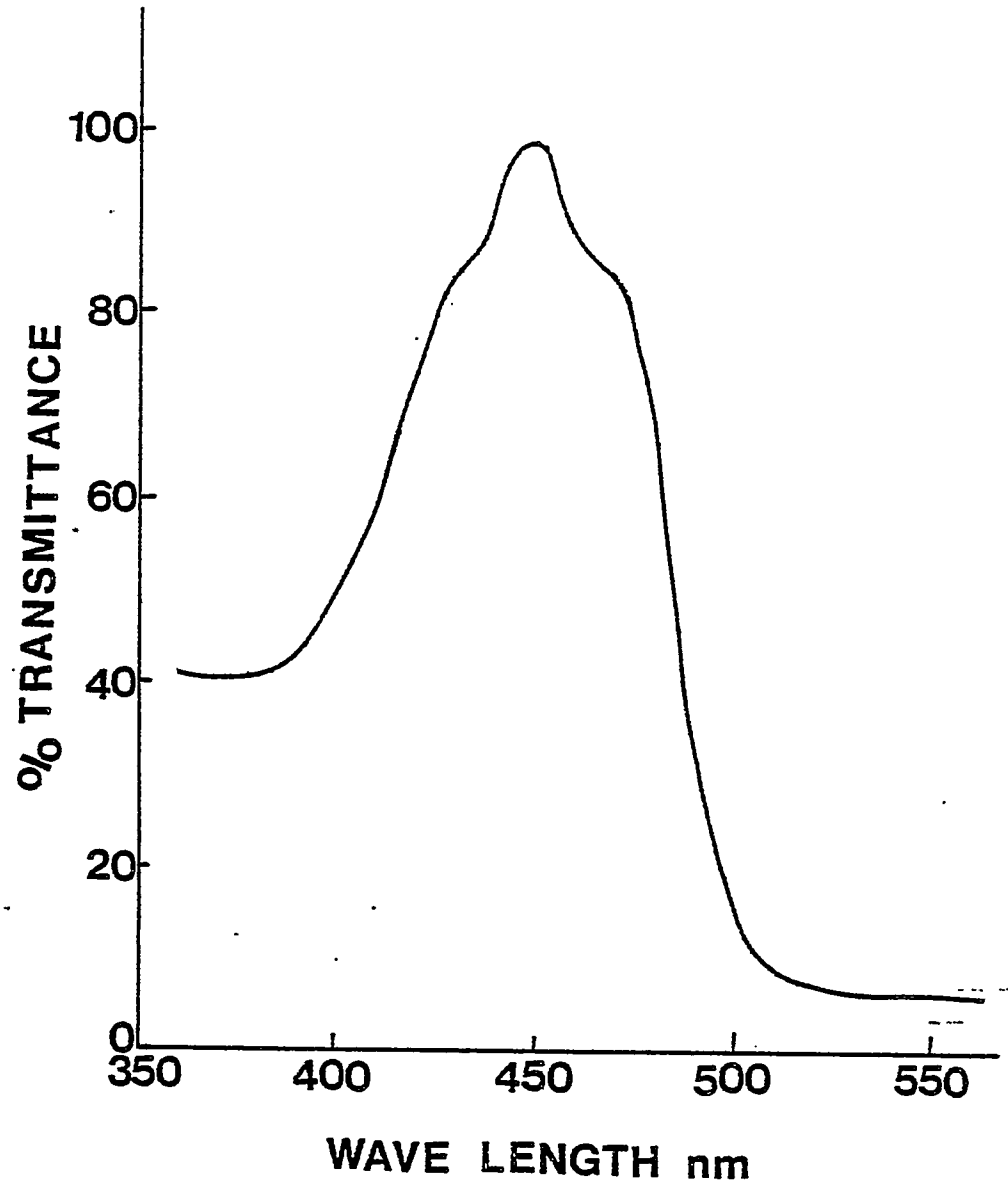


Figure 6. Absorption spectrum of β-carotene in chloroform.

Absorption spectrum (350 to 550 nm) of a 2.0 ml sample of 5.15×10^{-3} mg β-carotene/ml in a 1-cm light path.

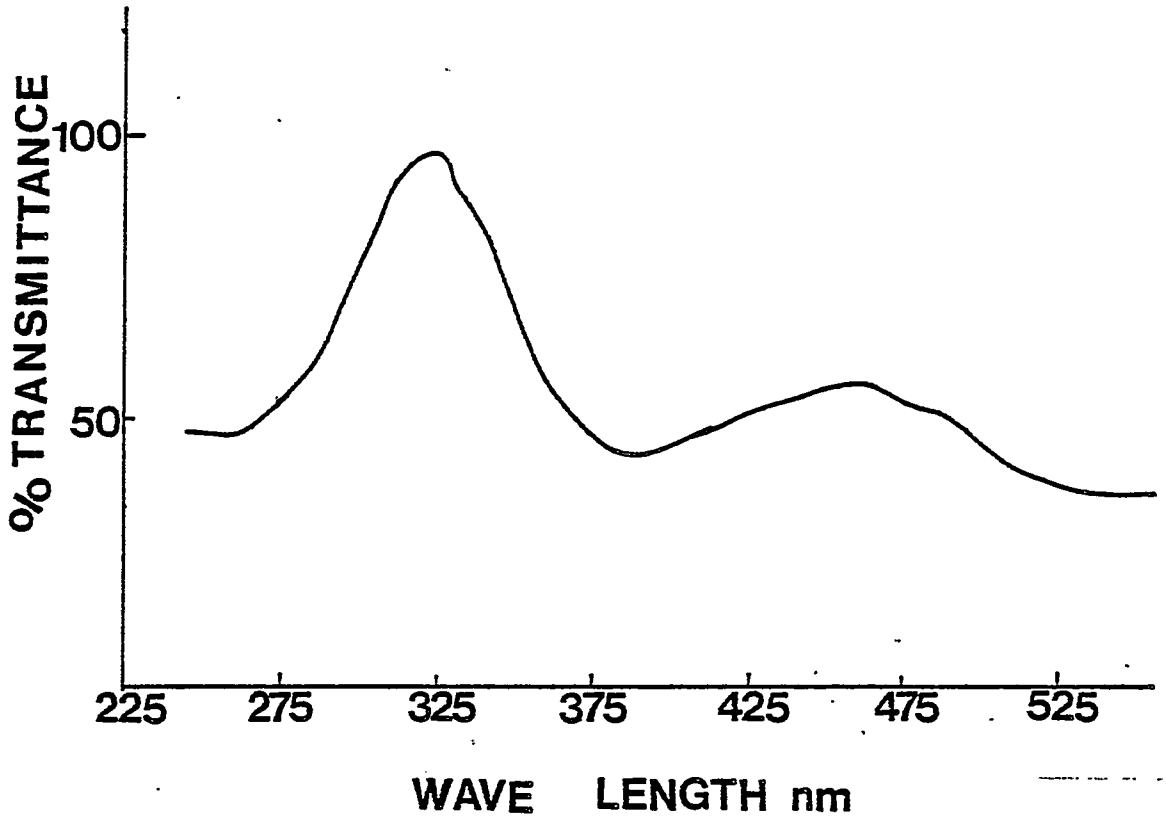


Figure 7. Absorption spectrum of vitamin A–β-carotene mixture in chloroform.

Absorption spectrum (225 to 550 nm) of a 1.0 ml sample of 1.6×10^{-3} mg vitamin A/ml plus a 1.0 ml sample of 5.15×10^{-3} mg β-carotene/ml in a 1-cm light path.

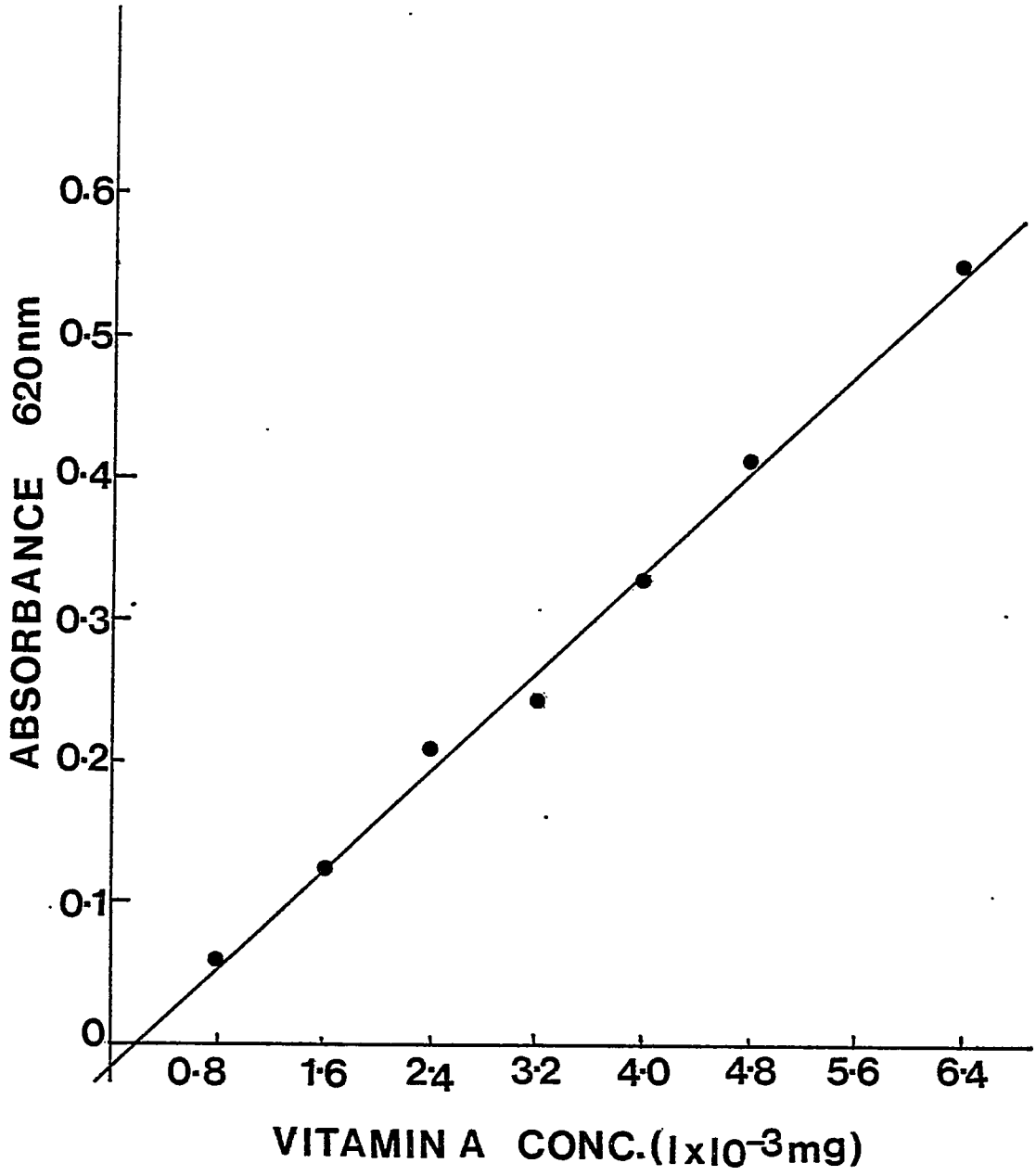


Figure 8. Standard curve for vitamin A.

Absorbance readings of several aliquots (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 ml) of 1.6×10^{-3} mg vitamin A/ml were taken following procedures described in Materials and Methods. The following is the equation of the resulting curve:

$$Y = -0.009 + 0.13799x$$

$$r = 0.999$$

Each point is a mean of 3 samples.

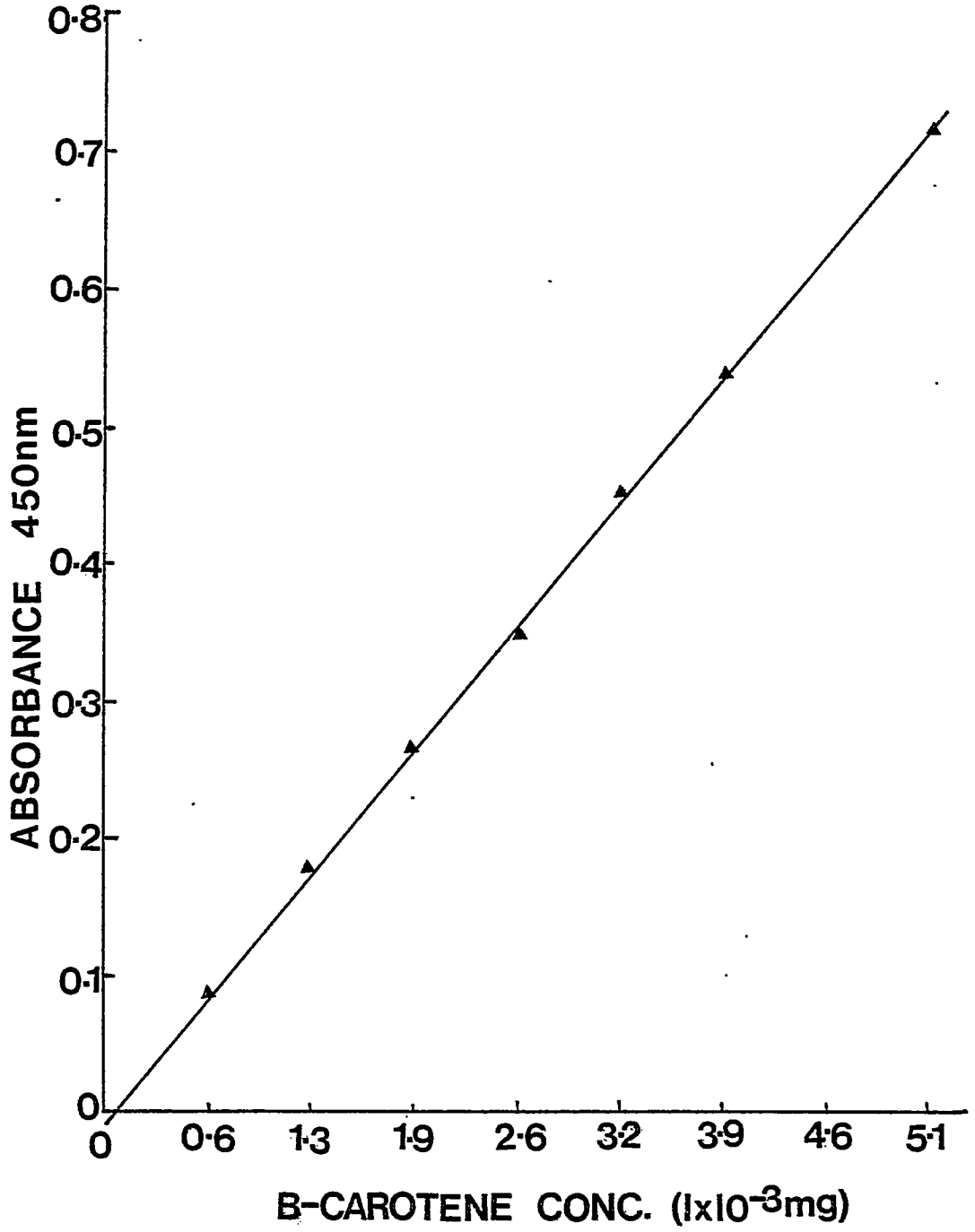


Figure 9. Standard curve for β-carotene.

Absorbance readings of several aliquots (0.125, 0.25, 0.375, 0.5, 0.625, 0.75, 1.0 ml) of 5.15 x 10^{-3} mg β-carotene/ml were taken following procedures described in Materials and Methods. The following is the equation of the resulting curve:

$Y = -0.006 + 0.7299x$

$r = 0.998$

Each point is a mean of 3 samples.

4.3 Wavelength effect on photodegradation of vitamin A

Maximal photodegradation of vitamin A occurred in the 340-440 nm range (Table 3). Longer wavelengths had little deleterious effects. The rate of vitamin A destruction by light increased with increasing exposure time (Table 3). The average loss in vitamin A potency upon 10 minutes of exposure was 4.40% as compared to 10.07% at 20 minutes of exposure. The time effects were found to be significantly different ($P \leq 0.01$).

4.4 The "protective" effect of β-carotene on the light-induced destruction of vitamin A

β-carotene in chloroform effectively reduced the light-induced destruction of vitamin A in chloroform (Table 4). The protective effect increased with increasing concentrations of β-carotene (Table 4). A 5.72% loss in vitamin A potency was observed when the vitamin A solution was exposed to light alone. This percentage loss was reduced to 3.30% and 2.56% upon addition of one part and two parts β-carotene, respectively. The protective effect of β-carotene was significant ($P \leq 0.05$) at both concentrations.

4.5 Some factors affecting the vitamin A and β-carotene contents of milk

A number of factors that affect the vitamin A activity of milk were investigated.

4.5.1 Effect of pasteurization on the vitamin A and β-carotene contents of 3.25% B.F. (homogenized) milk

The effect of pasteurization temperatures on the vitamin A activity of milk was investigated for a period of one year. The vitamin A and β-carotene content of homogenized milk, prior to and after pasteurization, is shown in

Table 3

Wavelength effect (ultraviolet and visible range) on the light-induced decomposition of vitamin A in ethyl ether, exposed for different periods of time.

Wavelength (nm)	Percent loss of original vitamin A concentration[1]	
	10-minute exposure	20-minute exposure
260	1.30	7.35
280	1.30	8.44
300	2.17	6.95
320	3.69	8.47
340	4.08	14.81
360	6.04	13.60
380	6.26	13.82
400	6.22	13.73
420	6.30	13.48
440	5.58	11.80
460	5.60	9.48
480	5.41	8.20
500	4.76	7.57
520	5.63	7.58
540	4.99	9.13
560	4.99	9.78
580	5.43	8.69
600	4.51	8.52
Mean	4.40	10.07

[1]absorbance technique

Table 4

"Protective" effects of β-carotene against photodegradation of vitamin A

Wavelength (nm)	Percent loss[1] of original concentration of vitamin A, after 10 minutes of exposure		
	Vitamin A	1:1 vitamin A: β-carotene mixture	1:2 vitamin A: β-carotene mixture
340	4.08	3.14	2.15
360	6.04	3.44	2.81
380	6.26	3.75	2.42
400	6.22	3.46	2.82
420	6.30	3.81	3.00
440	5.57	3.11	2.50
460	5.60	2.43	2.25
Mean	$5.72^{a,2}$	3.30^{b}	2.56^{b}

[1]Absorbance technique

[2]Means having the same superscript are not significantly different

Table 5. Pasteurized milk was found to contain 8.06% more vitamin A than unpasteurized milk. However, unpasteurized milk had 11.80% more β-carotene than pasteurized milk. These differences, however, were not found to be significant.

4.5.2 Effect of fortification on the vitamin A concentration in 2% B.F. and skim milk

The effect of fortification with synthetic vitamin A on the vitamin A concentration in 2% B.F. and skim milk was studied for a period of one year. The vitamin A content of unfortified 2% B.F. and skim milk and fortified 2% B.F. and skim milk are shown in Table 6.

The process of removing fat from milk is accompanied by a loss of vitamin A. As a result, partially skimmed or wholly skimmed milk have low vitamin A levels. The vitamin A content of the unfortified 2% B.F. milk (Table 6 and Figure 10) was found to be low with little seasonal fluctuations. The vitamin A content of skimmed milk (wholly or partially skimmed) can be replaced by the fortification with pure synthetic vitamin A. Fortified 2% B.F. milk was found to contain, on the average, 137% more vitamin A than the unfortified sample of the same B.F. percent. Fortified skim milk had 15 times as much vitamin A as unfortified skim milk. The vitamin A content of fortified skim (Table 6) and fortified 2% B.F. milk (Table 6, Figure 10) was found to fluctuate greatly from month to month.

4.5.3 Effect of storage (time and temperature) on the vitamin A content of milk with varying B.F. percent

The effects of a 21-day storage period, at two storage temperatures, on the vitamin A concentration in the milk of varying B.F. %, are shown

Table 5

Effect of pasteurization on the vitamin A and β-carotene contents of 3.25% B.F. milk.

Month of sample collection	Vitamin A concentration in the milk[1]		β-carotene concentration in the milk[1]	
	Unpasteurized[2]	Pasteurized[3]	Unpasteurized[2]	Pasteurized[3]
December	91.68	97.08	13.17	12.23
January	93.53	95.65	16.93	16.46
February	90.33	115.95	16.93	15.99
March	107.86	111.90	18.34	21.64
April	114.60	117.30	21.63	17.40
May	122.69	124.21	28.10	18.00
June	121.34	126.74	32.00	19.00
July	----	----	---	---
August	80.81	111.90	17.40	23.52
September	115.00	111.90	25.14	23.04
October	98.42	111.90	15.52	15.05
November	91.68	94.38	10.82	10.82
Mean ± sd[4]	102.54±14.3	110.81±10.93	19.63±6.45	17.56±4.1

[1]Vitamin A and β-carotene concentration expressed as IU/100 ml.

[2]Unpasteurized milk taken off the separator.

[3]Pasteurized milk taken after processing.

[4]Mean ± standard deviation of the samples.

Table 6

Effect of fortification on the vitamin A content of 2% B.F. and skim milk

Month of sample collection	Vitamin A concentration in the milk[1]			
	2% B.F. milk		Skim milk	
	No Vitamin A added[2]	Vitamin A added[3]	No Vitamin A added[2]	Vitamin A added[3]
December	66.04	229.20	--	167.18
January	76.94	79.55	--	105.46
February	76.94	102.45	--	225.10
March	71.48	203.59	9.44	217.07
April	80.59	190.10	--	94.38
May	91.13	151.10	--	91.68
June	93.07	288.43	--	223.81
July	87.64	101.12	15.01	----
August	86.29	245.38	--	----
September	87.64	237.29	--	232.30
October	90.33	289.87	12.54	221.10
November	78.33	217.07	--	217.07
Mean ± sd[4]	82.20±8.47	194.60±71.63	12.33±2.28	179.51±56.55

[1]Vitamin A concentration expressed as IU/100 ml.

[2]Samples were taken from the separator before vitamin A was added.

[3]Samples taken after pasteurization, before marketing.

[4]Mean ± standard deviation of the samples.

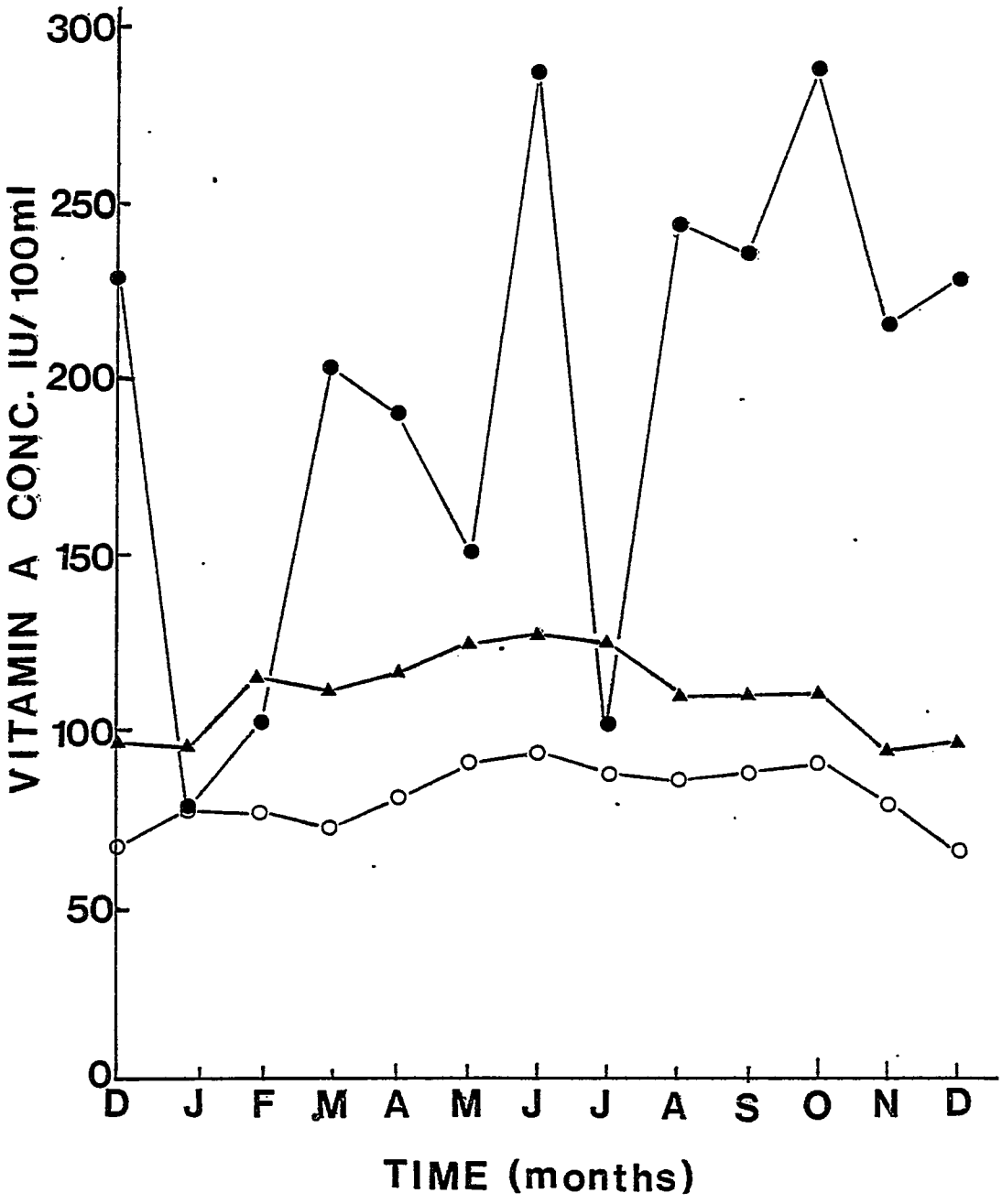


Figure 10. Effect of skimming followed by fortification on the vitamin A content of commercial milk.

The vitamin A content (IU/100 ml) of homogenized 3.25% B.F. milk (▲), unfortified 2% B.F. milk (○) and fortified (150 IU vitamin A/100 ml added) 2% B.F. milk (●) was determined monthly for a period of one year (June 1977 - June 1978).

in Table 7. Loss of vitamin A increased with increasing storage period in nearly all the samples, at both 15°C and -15°C. The pH readings taken at each analysis period indicated an increasing acidity in the milk. Milk samples stored in the freezer (-15°C) had significantly less vitamin A loss (57% less) than the samples stored in the refrigerator (15°C). The final percentage loss of vitamin A from skim, partially skimmed and homogenized milk was greater than that from whole milk (Table 7).

4.5.4 Comparison of the vitamin A activity of milk from the fluid milk tank and industrial silo

The vitamin A activity of monthly samples taken from the commercial fluid milk tank and industrial silo are shown in Table 8. Milk from the commercial fluid tank had 3.33% more vitamin A than industrial silo milk. No significant differences were observed between the vitamin A activity of milk from the fluid tank and the industrial silo. The vitamin A activity of fluid and industrial silo milk was found to be highest in the summer months and lowest in the winter months (Table 8).

4.5.5 Effect of time of milking (morning and afternoon) on the vitamin A content of milk

The effect of the time of milking on the vitamin A content of milk was determined (Table 9). The vitamin A content of afternoon milk had 28% more ($P \leq 0.05$) vitamin A potency (IU/100 ml) than that of the morning milk. However, when expressed on the basis of IU/gm B.F. or IU/milking, the vitamin A contents of morning and afternoon milk were not significantly different. The observation that the afternoon milk has more vitamin A than the morning milk requires that a composite sample of morning and afternoon milk be used in vitamin A assays.

Table 7

Effect of storage (time and temperature) on the vitamin A content of milk of varying B.F.%.

Storage Temperature	Days of Storage[2]	Vitamin A concentration in the milk[1]			
		Fortified skim milk[3] (.05% B.F.)	Fortified 2% B.F.[3] milk (1.84% B.F.)	Homogenized[3] milk (3.13% B.F.)	Whole[4] milk (3.88% B.F.)
Refrigerator (+5°C)	0	210.32	192.80	110.55	94.34
	7	208.40	190.56	103.81	95.72
	14	202.23	182.01	99.77	96.44
	21	178.5	179.31	95.72	91.68
	Final loss[5] (%)	17.9	7.52	15.00	2.90
Freezer (-15°C)	0	210.32	192.80	110.55	94.34
	7	209.07	193.01	105.16	95.72
	14	206.26	186.06	106.50	94.16
	21	194.40	180.67	88.42	93.00
	Final loss[5] (%)	8.18	6.71	11.19	1.44

[1]Vitamin A concentration expressed as IU/100 ml. Each value is a mean of two samples.

[2]Aliquots were taken from the main storage sample for assay at the specified storage period.

[3]Samples taken after processing, before marketing.

[4]Sample taken from the University dairy herd tank.

[5]Final loss of vitamin A potency expressed as percent of original potency.

Table 8

Comparison of the vitamin A content of milk from the fluid tank and the industrial silo.

Month of sample collection	Vitamin A concentration in the milk[1]	
	Fluid milk tank[2]	Industrial silo[3]
January	---	90.25
February	86.29	83.59
March	113.25	95.73
April	119.90	114.60
May	117.31	115.90
June	113.25	115.95
July	115.95	---
August	87.63	84.94
September	105.16	111.90
October	112.34	111.90
November	94.37	84.94
December	86.28	97.08
Mean ± sd[4]	103.97 ± 12.7	100.62 ± 13.57

[1]Vitamin A concentration expressed as IU/100 ml.

[2]Milk for fluid consumption - a collective sample from the entire Saskatoon milk-shed.

[3]Milk for industrial purposes - a collective sample from the entire Saskatoon milk-shed.

[4]Mean ± standard deviation of the samples.

Table 9

Effect of time of milking on the vitamin A content of milk.

Time of milking	Day of sample collection	Vitamin A concentration in milk[1]		
		IU/100 ml	IU/gm B.F.	IU/milking[2]
Morning	1	109.88	34.26	20104.75
	2	99.43	33.82	16894.00
	3	108.53	32.99	18610.25
	Mean ± sd[3]	105.94±5.68	33.69±0.64	18536.0±1604.8
Afternoon	1	124.10	36.10	17602.5
	2	145.83	38.78	21308.0
	3	138.19	34.74	18947.7
	Mean ± sd[3]	136.04±11.02	36.54±2.05	19286.0±1157.9

[1]Each value is a mean of four samples taken from four different cows.

[2]IU/milking equals the amount of vitamin A in the total volume of milk at one milking.

[3]Mean ± standard deviation of the samples.

4.5.6 Seasonal variations in the vitamin A and β-carotene contents of Saskatoon market milk

The vitamin A and β-carotene contents of Saskatoon market milk were obtained from five representative areas in the Saskatoon milk-shed during the period between June 1977 to June 1978. Monthly and seasonal levels of vitamin A and β-carotene in the market milk were determined (Tables 10, 11 and 12). Results are expressed as monthly and seasonal mean values of milk samples drawn from the five regions around Saskatoon.

The vitamin A and β-carotene content of milk rose sharply in the months of March and April, reaching a maximum of 136 IU vitamin A per 100 ml and 33 IU β-carotene per 100 ml, in the month of May (Figure 11). Vitamin A activity of summer milk declined throughout the months of June and July, reaching a minimum of 97 IU vitamin A per 100 ml and 17 IU β-carotene per 100 ml, in the month of August. The vitamin A activity of the milk rose slightly in the months of September and October, reaching an average of 110.3 IU vitamin A per 100 ml. The vitamin A activity of November milk was low (97.10 IU/100 ml). This level was maintained through the winter months of December, January and February (Figure 11). The vitamin A activity of the monthly and seasonal samples, when expressed as IU per gm B.F., showed the same seasonal trend as described above (Tables 10 and 11). The total potency of vitamin A (Table 12) also showed pronounced seasonal fluctuations. The butterfat percentage of the milk samples (Table 13) experienced little seasonal fluctuations.

The twelve months were grouped into four seasons, with winter comprising December, January and February; spring: March, April and May; summer: June, July and August and fall: September, October and November.

Table 10

Seasonal variations in the vitamin A content of Saskatoon market milk

Month of the year	Vitamin A concentration in the milk: Mean monthly values[1] IU/100 ml	Mean monthly values[1] IU/gm B.F.	Season of the year	Mean seasonal values[2] IU/100 ml	Mean seasonal values[2] IU/gm B.F.
December	100.84	27.60			
January	98.15	28.74	winter	99.40^{a} ±1.07[3]	28.38±0.39
February	99.23	28.79			
March	119.06	32.84			
April	120.53	33.91	spring	125.34^{b} ±5.56	31.50±2.81
May	136.44	38.60			
June	127.03	34.91			
July	120.66	33.70	summer	115.10^{ab} ±8.94	31.50±2.81
August	97.60	25.94			
September	110.56	30.47			
October	110.30	30.79	fall	106.00^{a} ±4.43	29.56±1.07
November	97.13	27.43			

[1]Each value is a mean of 5 samples from 5 different areas of the Saskatoon-milk shed.

[2]Each value is a mean of 15 samples ± standard error of the mean of seasonal samples.

[3]Means sharing the same superscript are not significantly different.

Table 11

Seasonal variations in the β-carotene content of Saskatoon market milk

Month of the year	β-carotene concentration in the milk: Mean monthly values[1] IU/100 ml	IU/gm B.F.	Season of the year	Mean seasonal values[2] IU/100 ml	IU/gm B.F.
December	14.11	4.06			
January	14.76	4.49	winter	14.92^{a} ±0.52[3]	4.47±0.23
February	15.90	4.86			
March	17.69	4.93			
April	25.02	7.07	spring	25.24^{b} ±4.42	7.16±1.31
May	33.00	9.47			
June	29.00	7.96			
July	25.51	5.15	summer	$24.25^{a,b}$ ±3.17	6.89±0.88
August	18.25	7.55			
September	20.69	5.64			
October	15.71	4.58	fall	$17.49^{a,b}$ ±1.60	4.87±0.39
November	16.08	4.59			

[1]Each value is a mean of 5 samples from 5 different areas of the Saskatoon-milk shed.

[2]Each value is a mean of 15 samples ± standard error of the mean of seasonal values.

[3]Means sharing the same superscript are not significantly different.

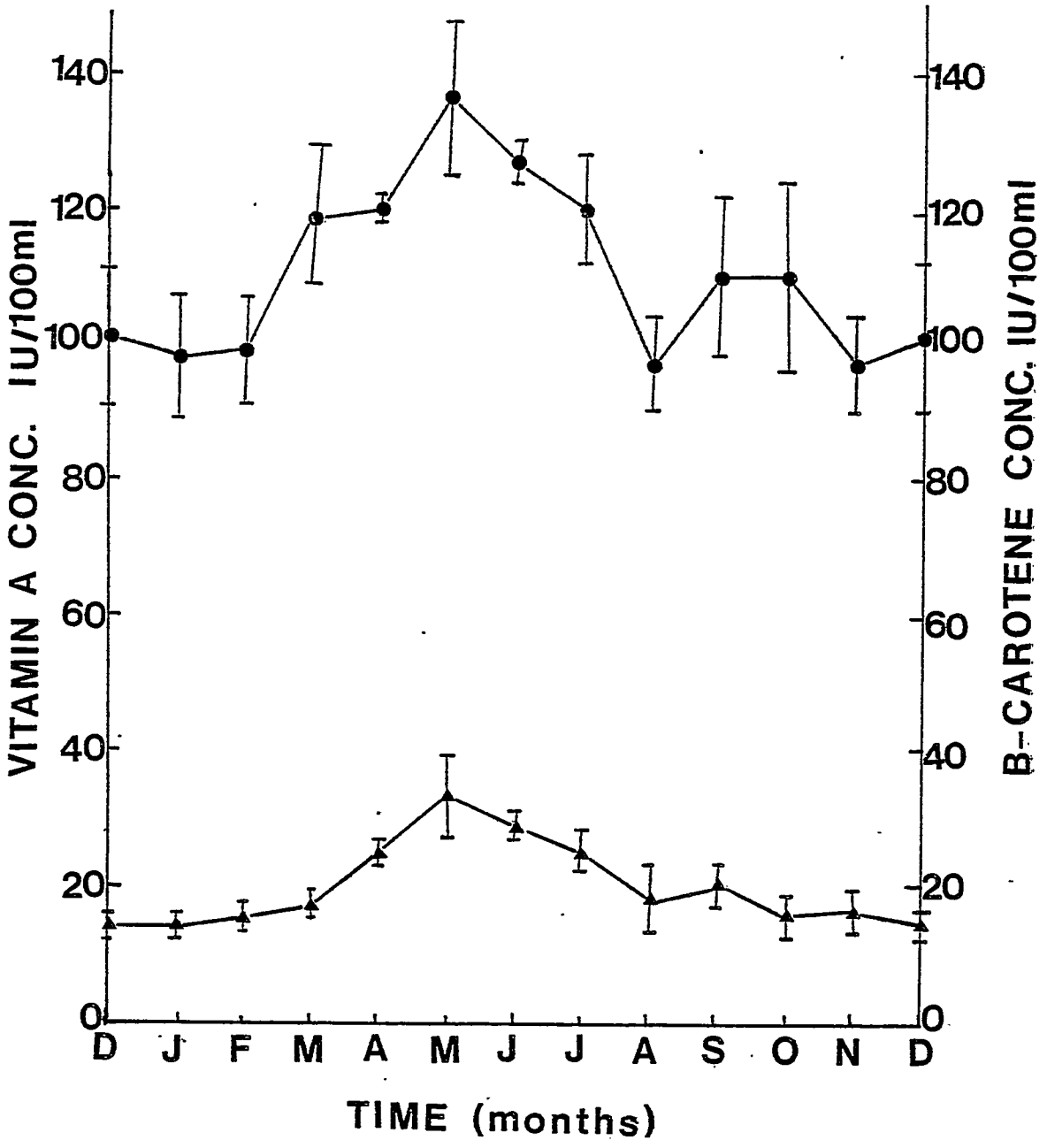


Figure 11. Seasonal variations in the vitamin A and β-carotene contents of Saskatoon fluid market milks.

The vitamin A (●) and β-carotene (▲) contents of monthly milk samples from 5 representative areas in the Saskatoon milkshed were determined for a period of one year (June 1977 - June 1978) and the values were expressed as IU/100 ml. Each point is a mean of 5 samples. Vertical bars indicate standard deviation from the mean.

Table 12

Seasonal variations in the total potency (vitamin A and β-carotene) of Saskatoon market milk.

Month of the year	Total vitamin A potency of the milk[1] Mean monthly values[2] IU/100 ml	IU/gm B.F.	Season of the year	Mean seasonal values[3] IU/100 ml	IU/gm B.F.
December	104.37	28.61			
January	101.84	29.86	winter	$103.14^{a} \pm 1.27^{4,5}$	29.49±0.76
February	103.21	30.00			
March					
March	123.48	34.07			
April	126.91	35.68	spring	131.69^{c} ±11.38	36.91±3.60
May	144.69	40.97			
June					
June	134.28	36.90			
July	127.04	34.99	summer	$121.16^{b,c}$±16.85	33.23±4.79
August	102.16	27.81			
September					
September	115.73	31.61			
October	114.23	31.93	fall	$110.37^{a,b}$±8.02	30.71±1.85
November	101.15	28.58			

[1]Values calculated from table 10 and table 11 using the fact that β-carotene has only 25% the activity of vitamin A for humans (NRC, 1971).

[2]Each value is a mean of 5 samples from 5 different areas of Saskatoon.

[3]Each value is a mean of 15 samples.

[4]± standard deviation of the samples.

[5]Means sharing the same superscript are not significantly different.

Table 13

Seasonal effects on the B.F.% of milk

Month of the year	Mean monthly B.F.%[1]	Season of the year	Mean seasonal B.F.%[2]
December	3.54		
January	3.29	winter	3.42±0.07
February	3.44		
March	3.57		
April	3.18	spring	3.39±0.11
May	3.43		
June	3.46		
July	3.42	summer	3.52±0.06
August	3.52		
September	3.63		
October	3.58	fall	3.58±0.03
November	3.53		

[1]Each value is a mean of 5 samples from 5 different areas of the Saskatoon-milk-shed.

[2]Each value is a mean of 15 samples ± standard error of the means of seasonal values.

Spring milk was found to contain the highest and winter milk the lowest concentration of vitamin A and β-carotene among the four seasons. The spring milk had 26%, 18.25% and 8.17% more vitamin A than winter, fall and summer milk, respectively. Summer milk contained 15.79% and 8.58% more vitamin A than that of winter and fall milk, respectively. Fall milk had 6.22% more vitamin A than winter milk.

The β-carotene content of spring milk was 69%, 44.25% and 4% higher than that of winter, fall and summer milk, respectively. Summer milk had a β-carotene content 62.60% and 33.70% higher than that of winter and fall milk, respectively. Fall milk was found to contain 17.20% more β-carotene than winter milk.

Statistical analysis revealed that spring milk had a significantly higher amounts ($P \leq 0.05$) of vitamin A and β-carotene (IU/100 ml) than winter and fall milk. The vitamin A potency of spring and summer milks were not significantly different. The vitamin A and β-carotene contents of the spring and summer milks varied greatly, as evidenced by the high standard error of the means (Tables 10 and 11). Winter milk had the least variable vitamin A concentration.

Seasonal variations were reduced upon expression of the vitamin A potency of the milk samples as IU/gm B.F. The differences in the vitamin A content of milk from the 5 different areas around Saskatoon (Table 14), were not significant.

A comparison of the commercial and the University herd milk (Figure 11) indicated that the vitamin A content of the latter did not show the seasonal variations shown by the former.

Table 14

Variations in the vitamin A content of Saskatoon market milk due to differences in areas of sample collection

Month of the year	Concentration of vitamin A in the milk[1]				
	Route 1A (Rosetown)	Route 2A (Warman)	Route 3A (Waldheim)	Route 4A (Viscount)	Route 5A (Asquith)
December	106.51	91.68	79.55	107.89	99.77
January	109.21	106.50	86.29	97.07	91.68
February	97.07	101.12	102.12	109.99	86.29
March	---	107.86	129.43	119.99	---
April	121.34	118.64	124.04	118.65	119.99
May	130.78	136.17	156.04	132.13	126.74
June	125.99	122.72	132.01	124.25	130.18
July	123.36	---	109.88	130.76	118.65
August	109.21	97.38	99.71	88.98	95.72
September	103.83	110.56	128.08	114.60	95.72
October	110.56	111.23	133.48	94.38	101.87
November	93.03	90.33	109.20	97.07	95.72
Mean ± sd[2]	111.9±12.09	108.56±13.70	115.82±21.97	111.31±14.53	110.92±13.95

[1]Vitamin A concentration expressed as IU/100 ml.

[2]Mean ± standard deviation of the samples.

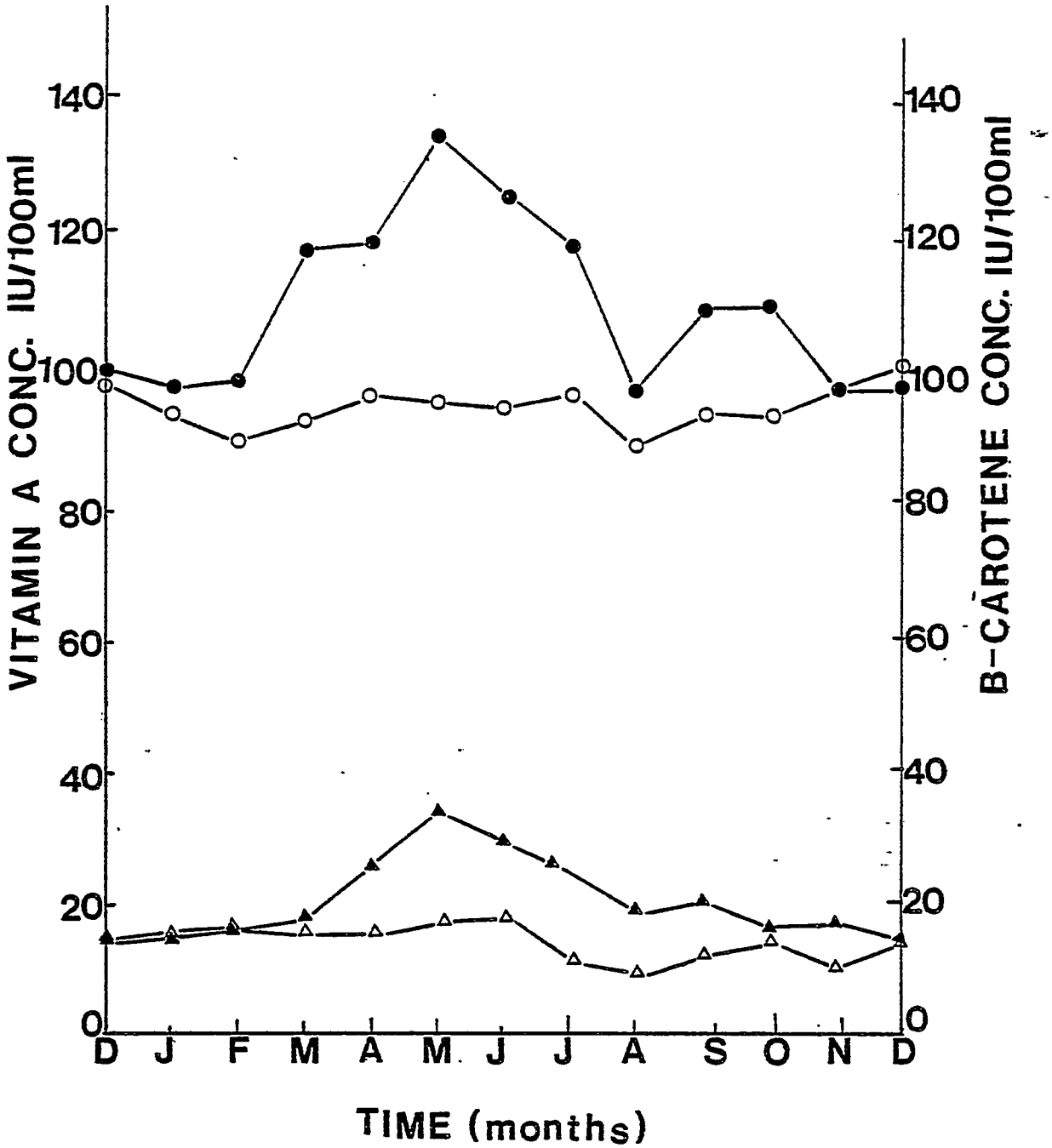


Figure 12. Comparison of seasonal variations in the vitamin A and β-carotene contents of commercial and University of Saskatchewan Dairy Herds.

The vitamin A content of monthly milk samples from commercial (●) and University (○) dairy herds and the β-carotene content of milk samples from commercial (▲) and University (△) dairy herds was determined for a period of one year (June 1977 - June 1978). The values were expressed as IU/100 ml.

4.5.7 Effect of increasing the vitamin A level of feed on the vitamin A content of milk of cows in two stages of lactation

The effect of increasing the vitamin A level in the feed on the vitamin A content of the resulting milk of cows in two stages of lactation was investigated in a 26-day experiment. The experimental feeds were: Feed I (8,200 IU vitamin A per kg feed); Feed II (87,600 IU vitamin A per kg feed) and Feed III (8,200 IU vitamin A per kg feed). The vitamin A content of the milk rose sharply upon increasing the vitamin A level in the feed from 8,200 IU per kg feed to 87,600 IU per kg feed (Table 15). Peak levels were obtained three to four days subsequent to the vitamin A addition (Figure 13) and were maintained for the duration of the supplementation. Upon removal of the extra dietary vitamin A, milk levels of the vitamin rapidly declined, within two days, to the original levels.

The vitamin A content of the milk from cows receiving Feed II was found to be 24% and 15% higher than that of the milk of cows receiving feeds I and III, respectively. Statistical analysis revealed that Feed II resulted in milk with a significantly higher ($P \leq 0.01$) vitamin A content than either Feed I or Feed III. Although the vitamin A content of milk of cows receiving Feed III was higher than that of cows on Feed I, the differences were not significantly different. The β-carotene content of the milk was not affected by the supplementation of feeds with vitamin A.

The milk of cows in late lactation had a vitamin A potency (IU/100 ml) significantly higher ($P \leq 0.05$) than the milk of cows in early lactation. However, when expressed as IU vitamin A per gm B.F., the milk of cows in early lactation had significantly a higher ($P \leq 0.05$) vitamin A content than that of cows in late lactation. Upon expression of the vitamin A

Table 15

Effect of increasing the vitamin A level of feed on the vitamin A content of milk of cows in two stages of lactation

Vitamin A concentration in the feed	Vitamin A concentration in the milk								
	IU/100 ml			IU/gm B.F.			IU/day		
	Early lactation	Late lactation	Mean[3] ± SEM[4]	Early lactation	Late lactation	Mean[3] ± SEM[4]	Early lactation	Late lactation	Mean[3] ± SEM[4]
Feed I 8,200 IU/ kg feed	83.53[1]	86.67[1]	85.10[a] ±1.57	26.20[1]	23.75[1]	24.93[a] ±1.23	20497[1]	20035[1]	20266[a] ±231
Feed II 87,600 IU/ kg feed	100.30[1]	110.82[1]	105.56[b] ±5.26	35.40[1]	30.54[1]	31.76[b] ±1.22	25701[1]	25412[1]	25556[b] ±144
Feed III 8,200 IU/ kg feed	86.78[2]	96.48[2]	91.63[a] ±4.85	27.32[2]	25.30[2]	26.31[a] ±1.01	22180[2]	20741[2]	21460[a] ±719
Mean ±SEM[4]	90.20 ±5.13	97.99 ±7.01		29.64 ±4.44	26.53 ±2.47		22972 ±1532	22062 ±1687	

[1]Each value is a mean of 25 samples (5 cows milked for 5 days).

[2]Each value is a mean of 15 samples (5 cows milked for 3 days).

[3]Means sharing the same superscript in the same column are not significantly different.

[4]± standard error of the mean

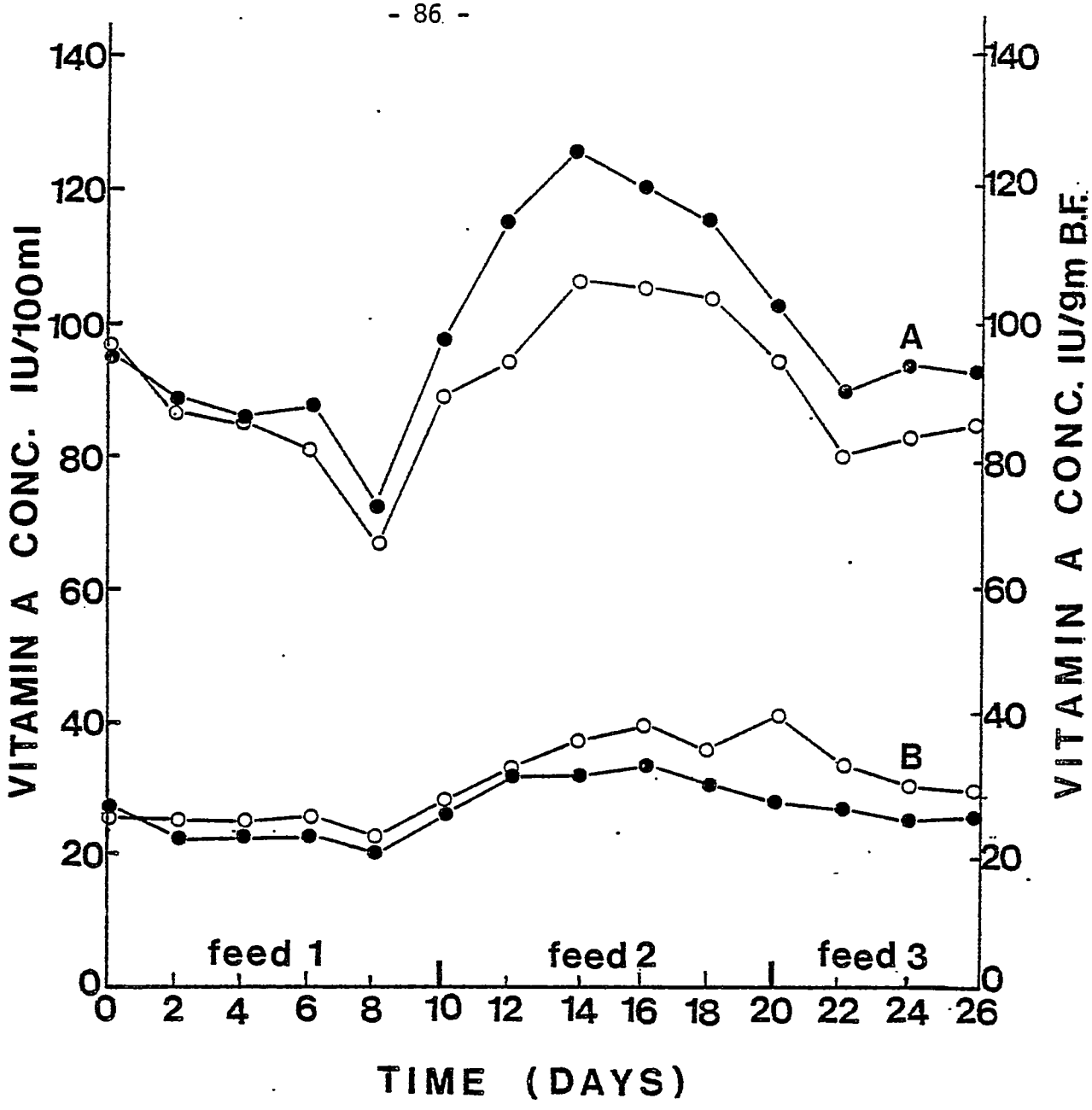


Figure 13. Effect of increasing the vitamin A level in the feed on the vitamin A content of milk of cows in two stages of lactation.

The vitamin A content of milk of cows in early (σ) and late (●) lactation, expressed as IU/100 ml (**A**) and IU/gm butter fat (**B**) was determined every other day for a period of 26 days. The cows were receiving 8,200, 87,600 and 8,200 IU vitamin A/kg feed on days 1-10, 10-20 and 20-26, respectively. Each point is the mean of 5 samples.

potency of the milk as IU per day, the stage of lactation had no significant effect.

Variations among cows in the vitamin A potency of the milk (IU/100 ml) (Table 16) were highly significant ($P \leq 0.01$) at the high level of supplementation for both early and late lactation cows. However, at the lower level of supplementation, the cow-to-cow variations in vitamin A potency of the milk were only significantly different for cows in late lactation.

The daily variations in the vitamin A potency of the milk (IU/100 ml) (Table 17) were highly significant ($P \leq 0.01$) at the low level of supplementation for early lactation cows. Upon extra supplementation, the daily variations (Table 17) in the vitamin A potency of the milk were not significantly different for both early and late lactation cows.

Milk yield (Table 18) of cows in late lactation was lower than that of cows in early lactation. Late lactation cows, however, produced milk with higher B.F. percent (Table 18). The level of vitamin A in the feed had no effect on either milk yield or B.F. percent.

4.5.8 Effect of supplementation of silage and hay with two levels of vitamin A on the vitamin A and β-carotene contents of milk, blood and fecal samples of the experimental cows

A 4 x 4 Latin square experiment was conducted in which four Holstein-Friesian cows, in the third and fourth months of lactation, were fed hay and silage supplemented with two levels of vitamin A. The lower level of supplementation consisted of hay or silage plus a calculated amount of high vitamin A concentrate so that the total vitamin A potency was approximately 250,000 IU/cow/day. The higher level of supplementation

Table 16

Within and among cow variations in vitamin A content of milk of cows in two stages of lactation, receiving two levels of dietary vitamin A

Stage of lactation	Cow number	Vitamin A concentration in the milk[1,2] Feed I (Mean ± sd)[3]	Feed II (Mean ± sd)
Early lactation	166	82.51±12.17	99.70± 9.73
	167	74.69± 8.64	82.70± 1.52
	165	91.68±13.40	109.20± 8.11
	156	76.04±12.19	94.00±12.54
	159	92.76±14.98	115.90±14.68
	Mean[4]±SEM[5]	83.54± 3.79	100.30± 5.77
	F	2.32	7.80**
Late lactation	104	105.40±10.44	140.40±14.10
	122	81.70± 7.54	79.50± 8.13
	121	83.50± 9.26	124.80±13.68
	115	77.90± 7.97	95.70±11.24
	69	84.85±11.27	113.70±11.32
	Mean[4]±SEM[5]	86.67± 4.83	110.82±10.70
	F	6.59**[6]	12.05**

[1]Vitamin A concentration in milk, expressed as IU/100 ml.

[2]Each value is a mean of 5 samples.

[3]Mean ± standard deviation of the samples.

[4]Overall mean, each is composed of 25 samples (5 cows milked for 5 days).

[5]Standard error of the overall mean.

[6]** Indicates significancy at the 99% level.

Table 17

Daily variation in the vitamin A content of milk of cows in two stages of lactation, receiving two levels of dietary vitamin A

Stage of lactation	Day	Vitamin A concentration in the milk[1,2] Feed I 8,200 IU vitamin A per kg feed	Feed II 87,600 IU vitamin A per kg feed
Early lactation	1	97.54	89.20
	2	86.24	94.32
	3	85.06	107.00
	4	81.56	106.46
	5	67.38	104.54
	Mean±SEM[3]	83.56±4.85	100.40±3.65
	F	5.32**[4]	1.54
Late lactation	1	95.46	98.38
	2	89.60	116.96
	3	86.26	126.42
	4	88.28	121.32
	5	73.91	111.06
	Mean±SEM[3]	86.70±3.55	113.80±5.85
	F	2.20	1.60

[1]Vitamin A concentration of milk, expressed as IU/100 ml.

[2]Each value is a mean of 5 samples from 5 different cows.

[3]Overall mean ± standard error of the mean. Each value is a mean of 25 samples.

[4]** Indicates significancy at 99% level.

Table 18

Effect of increasing the vitamin A level of feed on the yield and B.F.% of milk of cows in two stages of lactation

Concentration of vitamin A in the feed	Milk yield[3]		% B.F.	
	Early lactation	Late lactation	Early lactation	Late lactation
Feed I[1]	24.48	23.38	3.38	3.70
Feed II[1]	25.47	22.30	3.11	3.79
Feed III[2]	25.42	21.78	3.05	3.87

[1]Milk yields and %B.F. values for feeds I and II are a mean of 25 samples.

[2]Milk yields and %B.F. values for feed III are a mean of 15 samples.

[3]Milk yields are expressed as kg per day.

consisted of silage or hay plus a calculated amount of high-vitamin A concentrate so that the final vitamin A potency was approximately 600,000 IU/cow/day. An additional amount of a low-vitamin A concentrate was fed to each cow, as required, in order to meet NRC (1971) recommendations for energy and milk production.

The vitamin A content of the experimental feeds was analysed prior to the beginning of each experimental period. The results (Table 19) indicate that alfalfa hay had twice as much β-carotene as wheat silage. Rate of carotene loss from hay and silage, over the 4-month experimental period, was low. The high vitamin A concentrate had 15 times as much vitamin A as the low vitamin A concentrate. Daily feed intakes of the experimental cows and the subsequent vitamin A intake per cow per day are shown in Table 20.

4.5.8.1 Effect of supplementation of silage and hay on the vitamin A and β-carotene contents of milk

A. Vitamin A concentration in the milk

Milk samples were collected during the last three days of each experimental period and assayed for vitamin A. The vitamin A recovery in the milk, expressed on the basis of IU/100 ml, is shown in Table 21 A . Milk of high vitamin A-fed cows had a significantly higher ($P \leq 0.01$) vitamin A content (IU/100 ml) than the milk of low vitamin A-fed cows. At both levels of supplementation, silage-fed cows tended to produce milk having a higher vitamin A content than hay-fed cows. At the low level of supplementation, the milk of silage-fed cows contained 26.20% more vitamin A than the milk of hay-fed cows. The observed difference, however, was not significant. At the higher level of supplementation, silage-fed cows produced milk containing significantly ($P \leq 0.01$) more vitamin A (35%) than the milk of hay-

Table 19

The β-carotene and vitamin A content of the experimental feeds[1] (Dry matter)

Experimental period	Forages		Dairy Concentrate	
	Silage[2]	Hay[2]	#172[3]	#173[4]
I	28.08	58.04	5062	83633
II	30.01	59.50	6000	85400
III	29.75	58.61	3928	72335
IV	31.05	56.44	3373	82261
Mean	29.72	58.15	4590	80907

[1]The analyses for the β-carotene and vitamin A were carried out immediately prior to the commencement of each experimental period.

[2]Wheat silage was freeze dried to 42% dry matter and alfalfa hay was oven dried to 88% dry matter before the analysis. The values are expressed on dry matter basis, as mg β-carotene per kg feed. About 10 kg of each forage was fed per cow per day.

[3]Dairy Concentrate #172, the low-vitamin A concentrate. The values are expressed as IU/kg feed.

[4]Dairy Concentrate #173, the high-vitamin A concentrate. The values are expressed as IU/kg feed.

Table 20

Average feed intake of the experimental animals and the vitamin A equivalent of the feeds per cow per day

Cow number	Experimental period	Average feed intake[1] (kg feed/cow/day)					Vitamin A equivalent[7] of the feed/cow/day
		Forage		Concentrate		TDN[6]	
		Silage[2]	Hay[3]	#172[4]	#173[5]		
83	I H_1	-----	9.36	13.51	---	12.44	291,260
	II H_2	-----	9.91	5.85	3.9	11.17	609,868
	III S_1	10.59	----	7.43	1.6	12.44	270,942
	IV S_2	10.59	----	3.50	5.6	12.40	603,993
101	I H_2	-----	9.91	7.66	4.0	12.30	603,376
	II H_1	----	9.91	9.91	---	11.27	295,318
	III S_2	10.59	----	3.50	6.5	12.97	609,948
	IV S_1	10.59	----	5.85	1.5	11.40	274,650
115	I S_2	9.91	----	7.60	5.2	14.15	604,431
	II S_1	9.91	----	9.91	1.4	13.28	296,949
	III H_2	----	9.36	5.28	5.0	12.30	601,849
	IV H_1	----	9.36	10.81	---	12.14	247,713

Table 20 (continued)

Cow number	Experimental period	Average feed intake[1] (kg feed/cow/day)					Vitamin A equivalent[7] of the feed/cow/day
		Forage		Concentrate		TDN[6]	
		Silage[2]	Hay[3]	#172[4]	#173[5]		
174	I S_1	10.20	---	7.21	1.0	11.65	253,442
	II S_2	9.91	---	2.93	5.5	11.59	609,169
	III H_1	-----	9.36	7.21	---	9.38	247,736
	IV H_2	----	9.36	3.00	5.8	10.40	603.483

[1]Values calculated from dairy barn feeding sheets. Each value represents a mean of the last 10 days of each experimental period minus the feed weighback.

[2]Wheat silage, 42% DM (freeze dried), 67% TDN in DM.

[3]Alfalfa hay, 88% DM (oven dried), 55% TDN in DM.

[4]Dairy concentrate #172 low vitamin A concentrate fed to meet NRC (1971) requirements for energy and milk production.

[5]Dairy concentrate #173 high vitamin A concentrate fed to meet the required level of vitamin A.

[6]TDN calculated using values from feed composition values (section 3.4.8).

[7]Total vitamin A intake/day/cow calculated using values from Table 16. mg β-carotene was taken to be equivalent to 400 IU vitamin A (NRC, 1971). Vitamin A intake expressed as IU/cow/day.

Table 21

Effect of supplementation of hay and silage with two levels of vitamin A on the vitamin A concentration in milk

A. Vitamin A concentration of the milk expressed as IU/100 ml[1]

Period	Cow Number 115	101	83	174	Period means[2] ± SEM
I	S_2 200.89	H_2 196.40	H_1 119.99	S_1 132.58	162.46±21.07
II	S_1 93.48	H_1 100.22	H_2 163.59	S_2 194.15	137.86±24.52
III	H_2 176.62	S_2 293.92	S_1 139.77	H_1 102.47	178.19±41.44
IV	H_1 103.81	S_1 172.58	S_2 285.83	H_2 191.45	188.42±37.54
Cow means[2] ± SEM	143.70±26.56	190.78±40.00	177.29±37.26	155.16±22.59	166.73±11.01

Treatment means[2,3] ± SEM

S_1 (Low level vitamin A - silage)	134.60^a±16.24	S_2 (High level vitamin A - silage)	243.70^b±26.75
H_1 (Low level vitamin A - hay)	106.62^a± 4.52	H_2 (High level vitamin A - hay)	182.01^c± 7.44

[1]Each value is a mean of 3 samples.

[2]Mean ± standard error of the mean for 12 samples.

[3]Means sharing the same superscript are not significantly different.

fed cows.

The vitamin A content of the milk, expressed as IU/gm B.F. (Table 21 B), showed no significant differences between the treatments: H_1, H_2 and S_1. High vitamin A-silage (S_2), however, resulted in a vitamin A content of the milk that was significantly higher than the remainder of the feeds.

B. β-carotene concentration in the milk

The β-carotene content of the milk samples (IU β-carotene/100 ml) was analysed (Table 22). Dietary silage and hay had greater influence on the β-carotene content of milk at the higher level of supplementation. Although the addition of vitamin A to silage did not affect β-carotene secretion in the milk, the addition of the vitamin to hay appears to have decreased the β-carotene content of the resulting milk. The β-carotene content of the milk, expressed as IU/gm B.F., showed no significant treatment differences.

4.5.8.2 Effect of supplementation of hay and silage on the vitamin A and β-carotene levels in blood

The vitamin A concentration in the blood of cows fed vitamin A supplemented silage and hay was analysed (Table 23 A). No significant differences were observed in the vitamin A concentration of blood of cows fed the experimental feeds.

The β-carotene concentration in the blood of the experimental animals is shown in Table 23 B . More β-carotene was observed in the blood at low levels of supplementation (S_1 and H_1) than at the corresponding high levels of supplementation (S_2 and H_2).

Table 21 (continued)

B. Vitamin A concentration of the milk expressed as IU/gm B.F.[1]

Period	Cow Number 105	101	83	174	Period means[2] ± SEM
I	S_2 96.58	H_2 49.47	H_1 26.43	S_1 52.82	56.32±14.64
II	S_1 40.94	H_1 25.33	H_2 45.86	S_2 81.97	48.52±11.98
III	H_2 51.71	S_2 107.94	S_1 36.02	H_1 32.07	56.93±17.52
IV	H_1 37.95	S_1 43.73	S_2 67.59	H_2 62.42	52.92± 7.15
Cow means[2] ± SEM	56.79±13.59	56.62±17.87	43.97±8.81	57.32±10.37	53.63±3.24

Treatment means[2,3] ± SEM

S_1 43.38^a ± 3.53 S_2 88.52^b ± 8.77 H_1 30.44^a ± 2.90 H_2 52.36^a ± 3.56

[1]Each value is a mean of 3 samples.

[2]Mean and standard error of the mean for 12 samples.

[3]Means sharing the same superscript are not significantly different.

Table 22

Effect of supplementation of silage and hay with two levels of vitamin A on the β-carotene content of milk[1,2]

β-carotene Period	Cow Number 115	101	83	174	Period means[3] ± SEM
I	S_2 13.80	H_2 15.68	H_1 19.29	S_1 14.42	15.80±1.23
II	S_1 8.63	H_1 15.52	H_2 11.45	S_2 8.94	11.13±1.59
III	H_2 11.13	S_2 17.14	S_1 18.50	H_1 17.87	16.16±1.66
IV	H_1 20.23	S_1 23.68	S_2 23.83	H_2 20.39	22.03±1.00
Cow means[3] ± SEM	13.45±2.50	18.00±1.93	18.27±2.50	15.40±2.48	16.28±2.23

Treatment means[3,4] ± SEM

S_1 16.31^a ± 3.18 S_2 15.96^a ± 3.13 H_1 18.23^b ± 1.02 H_2 14.66^a ± 2.17

[1]Expressed as IU/100 ml.

[2]Each value is a mean of 3 samples.

[3]Mean ± standard error of the mean for 12 samples.

[4]Means sharing the same superscript are not significantly different.

Table 23

Effect of supplementation of silage and hay on the vitamin A and β-carotene content of blood

A. Vitamin A concentration of blood[1,2]

Period	Cow Number 115	101	83	174	Period means[3] ± SEM
I	S_2 460.65	H_2 292.15	H_1 397.74	S_1 361.78	378.07±35.18
II	S_1 306.73	H_1 296.62	H_2 438.18	S_2 424.70	366.56±37.62
III	H_2 423.01	S_2 492.12	S_1 412.90	H_1 584.80	478.21±39.65
IV	H_1 303.36	S_1 316.84	S_2 340.43	H_2 364.03	331.16±13.42
Cow means[3] ± SEM	373.44±40.23	349.42±47.86	397.31±20.80	433.83±52.40	388.50±31.53

Treatment means[3] ± SEM

S_1 349.56 ± 24.27 S_2 409.47 ± 32.72 H_1 395.60 ± 67.15 H_2 379.33 ± 33.18

[1]Vitamin A concentration expressed as IU/100 ml.

[2]Each value is a mean of 3 samples.

[3]Mean ± standard error of the mean for 12 samples.

Table 23 (continued)

B. β-carotene concentration of blood[1,2]

Period	Cow Number 115	101	83	174	Period means[3] ± SEM
I	S_2 141.11	H_2 179.33	H_1 514.48	S_1 373.95	302.22±87.21
II	S_1 98.78	H_1 275.17	H_2 308.69	S_2 168.16	212.70±48.37
III	H_2 210.50	S_2 162.28	S_1 345.73	H_1 646.77	341.32±108.97
IV	H_1 253.42	S_1 198.74	S_2 310.45	H_2 359.84	280.61±34.89
Cow means[3] ± SEM	175.95±34.60	203.88±24.90	369.84±48.96	387.18±98.44	284.21±26.95

Treatment means[3,4] ± SEM.

S_1 254.30^a ± 64.52 S_2 195.50^a ± 53.63 H_1 422.46^b ± 95.33 H_2 264.59^a ± 42.04

[1]β-carotene concentration is expressed as IU/100 ml.

[2]Each value is a mean of 3 samples.

[3]Mean ± standard error of the mean for 12 samples.

[4]Means sharing the same superscript are not significantly different.

4.5.8.3 Effect of supplementation of hay and silage on fecal vitamin A and β-carotene

The vitamin A and β-carotene contents of fecal samples are shown in Tables 24 A and 24 B , respectively. Data for period I was lost due to technical difficulties. On the whole, negligible amounts of the dietary vitamin A and β-carotene were recovered in the feces of the experimental animals. From the available data, fecal losses of β-carotene were greater than fecal losses of vitamin A.

Balance studies, using lignin as the non-absorbable reference standard, showed that the apparent digestibility of the β-carotene of the wheat silage and alfalfa hay forages used in the experiment were 98.81% and 97.46% respectively. Overall ration dry matter digestibility was 56.23%. Wing (1969) obtained apparent digestibility of 95.27%, 85.40% and 55.35% for β-carotene of hay, silage and for ration dry matter, respectively.

4.5.8.4 Effect of supplementation of silage and hay on the yield and B.F.% of milk

The milk yield of the experimental cows (Table 25 A) was affected by neither the level of vitamin A in the feed nor the type of forage fed. Silage-fed cows tended to produce milk with less B.F.% (Table 25 B) than hay-fed cows. The vitamin A level in the feed did not affect the B.F.% of the milk.

Table 24

Effect of vitamin A supplementation of hay and silage on the vitamin A and β-carotene content of fecal samples

A. Vitamin A content of fecal samples[1,2]

	Cow Number			
Period	115	101	83	174
I	S_2	H_2	H_1	S_1
	-	-	-	-
II	S_1	H_1	H_2	S_2
	13.75	23.58	22.64	10.92
III	H_2	S_2	S_1	H_1
	14.28	18.60	23.06	17.22
IV	H_1	S_1	S_2	H_2
	19.68	26.28	21.44	18.86

Treatment means[3] ± SEM.

S_1 14.36 ± 3.76
S_2 16.99 ± 3.40
H_1 20.16 ± 1.85
H_2 18.59 ± 2.42

[1]Expressed as IU/kg fresh fecal material.

[2]Each value is a mean of 3 samples.

[3]Mean ± standard error of the mean for 12 samples.

Table 24 (continued)

B. β-carotene content of fecal samples.[1,2]

	Cow Number			
Period	115	101	83	174
I	S_2	H_2	H_1	S_1
	-	-	-	-
II	S_1	H_1	H_2	S_2
	129.1	186.95	210.95	91.50
III	H_2	S_2	S_1	H_1
	227.65	228.85	266.25	259.15
IV	H_1	S_1	S_2	H_2
	196.40	292.60	245.95	258.25

Treatment means[3] ± SEM.

S_1 229.32 ± 50.68
S_2 188.76 ± 48.18
H_1 214.16 ± 22.66
H_2 232.28 ± 13.85

[1] β-carotene content expressed as IU/kg fresh fecal material.

[2] Each value is a mean of 3 samples.

[3] Mean ± standard error of the mean for 12 samples.

Table 25

Effect of supplementation of silage and hay with two levels of vitamin A on the yield and B.F. % of milk

A. Milk yield[1,2]

Period	Cow Number 115	101	83	174	Period means[3] ± SEM
I	S_2 34.95	H_2 28.05	H_1 29.56	S_1 28.51	30.27±1.57
II	S_1 33.50	H_1 24.60	H_2 25.20	S_2 30.90	28.55±2.18
III	H_2 30.60	S_2 18.70	S_1 16.47	H_1 25.13	22.72±3.20
IV	H_1 29.10	S_1 22.83	S_2 21.67	H_2 25.40	24.75±1.65
Cow means[3] ± SEM	32.04±1.33	23.54±1.94	23.22±2.77	27.48±1.37	26.57±1.73

Treatment means[3] ± SEM

S_1 25.33 ± 3.67 S_2 26.55 ± 3.82 H_1 27.31 ± 1.30 H_2 27.10 ± 1.27

[1]Milk yield expressed as kg/day.

[2]Each value is a mean of 3 samples.

[3]Mean ± standard error of the mean for 12 samples.

Table 25 (continued)

B. B.F.% of the milk[1]

Period	Cow Number 115	101	83	174	Period means[2] ± SEM
I	S_2 2.08	H_2 3.97	H_1 4.54	S_1 2.51	3.27±0.58
II	S_1 2.29	H_1 3.96	H_2 3.58	S_2 2.38	3.05±0.42
III	H_2 3.42	S_2 2.72	S_1 3.88	H_1 3.20	3.30±0.24
IV	H_1 2.82	S_1 3.94	S_2 4.24	H_2 3.07	3.52±0.34
Cow means[2] ± SEM	2.65±0.30	3.65±0.31	4.06±0.21	2.79±0.20	3.29±0.10

Treatment means[2] ± SEM.

S_1 3.16 ± .47 S_2 2.74 ± .48 H_1 3.63 ± .38 H_2 3.51 ± .19

[1]Each value is a mean of 3 samples.

[2]Mean ± standard error of the mean for 12 samples.

5. DISCUSSION

The present study was undertaken to investigate and determine the factors causing variations in the vitamin A and β-carotene contents of milk. The main factors are metabolism of the dairy cow, dietary intakes of the cow and the processing, marketing and handling of the milk prior to consumption. Alone or in combination, these factors affect the ultimate vitamin A potency of milk and milk products.

5.1 Effect of metabolism on the vitamin A and β-carotene contents of milk

The stage of lactation (Table 15) was observed to have significant ($P \leq 0.05$) effects on the vitamin A content of the milk, in contrast to reports of McGillivray (1960), Smith (1967) and Hartman and Dryden (1974). As the stage of lactation advances, milk yield decreases and the butter fat content of the milk increases (Table 18). Hence, late lactation cows produce milk containing more vitamin A per 100 ml while early lactation cows produce milk containing more vitamin A per gm butter fat. However, when expressed as IU per day (Table 15) the difference in the vitamin A content of milk from cows in early and late lactation is not significant. The effects of stage of lactation on the vitamin A content of milk were increased by increasing the vitamin A level in the feed of dairy cows (Table 15, Figure 13).

The vitamin A content of milk of cows in the same breed and in the late stages of lactation (Table 16) was found to be significantly different ($P \leq 0.01$) at dietary vitamin A levels of 8,200 and 87,600 IU vitamin A/kg feed. The vitamin A content of milk of early lactation cows, however, was

only significantly different ($P \leq 0.01$) when the cows were receiving 87,600 IU vitamin A/kg feed. Cow-to-cow variations were also reported by McGillivray (1960) who noted that pasture-fed cows of the same breed could produce milk having vitamin A values ranging from one-half to twice herd average values.

Day-to-day variations in the vitamin A content of milk (Table 17) were found to be significant only at the low level of vitamin A supplementation for cows in the early stages of lactation. The vitamin A content of milk of cows in late lactation showed no significant daily variations. McGillivray (1960) observed greater daily variations in the vitamin A content of milk of pasture-fed cows than that of stall-fed cows.

The milk obtained in the afternoon milking (Table 9) had significantly ($P \leq 0.05$) more vitamin A (IU/100 ml) than that in the milk obtained in the morning milk. The time of milking had no significant effects when the vitamin A content of the milk was expressed as either IU/gm butter fat or IU/total volume of milk at a single milking.

Stage of lactation variations, cow-to-cow variations, daily variations and variations due to the time of milking can significantly influence the vitamin A content of milk under the described conditions. These factors are classified as "uncontrolled" because they are neither under the influence of the producer nor the processer.

5.2 Effect of the diet on the vitamin A and β-carotene contents of milk

Variations in the carotene and vitamin A contents of the feed have the greatest influence on the vitamin A content of the milk.

5.2.1 Seasonal variations (variations in the β-carotene intake)

Season *per se* has no effect on the vitamin A potency of milk. The seasonal variations that are generally observed in the vitamin A potency of milk merely reflect the amount of β-carotene present in the feed at different times of the year. In fact, the vitamin A content of milk of stall-fed cows was found to be stable throughout the year (Figure 12) while that of pasture-fed cows fluctuated with the seasons (Figure 12). Upon examination of Saskatoon bulk tank samples for a period of one year, pronounced seasonal variations were observed in the vitamin A potency of the milk (Table 10, Figure 11). On the average, spring and summer milk contained 1.17 times more vitamin A than that of winter and fall milk. Hartman and Dryden (1974) had reported that summer milk can contain from 1.5 to 15 times more vitamin A than winter milk but explained that the smaller differences are more common except when winter feeds are extremely poor. Comparable seasonal variations in the vitamin A content of milk have been observed in other Canadian regions such as Alberta (Searles and Armstrong, 1970); pooled samples from Halifax, Toronto, Montreal, Winnipeg and Vancouver (Thompson et al., 1972) and Ontario and Manitoba (Hartman and Dryden, 1974) (Table 26). A comparison of Canada and other countries (Table 26) indicates generally higher vitamin A levels in summer and lower levels in winter milk for all the countries except New Zealand. In New Zealand, the green winter pastures ensure better grazing than the relatively dry summer pastures (McGillivray, 1960; Thompson, 1968) hence, winter milk has more vitamin A than summer milk.

The sudden increase in the vitamin A potency of Saskatoon market milk in March (Figure 11) could not be accounted for. Farmers could not have

Table 26

Comparison of summer and winter vitamin A values of milk from different countries

Country	vitamin A content[1] of milk	
	summer	winter
Canada		
Saskatoon[2]	35.02	28.38
Alberta[3]	30.50	22.60
Ontario[4]	66.20	44.60
Manitoba[4]	60.60	37.40
Halifax - Montreal - Toronto - Winnipeg - Vancouver[5]	45.55	31.11
USA		
Wisconsin[6]	41.50	20.00
New York[6]	35.00	14.50
Tennesse[6]	47.50	20.00
Denmark[7]	40.66	23.00
Great Britain[7]	40.66	28.30
Netherlands[4]	90.90	58.10
Sweden[7]	30.00	18.30
Holland[7]	45.60	29.00
New Zealand[7]	40.60	51.70

[1]All values are recalculated to IU/gm butter fat.

[2]Results of the present study.

[3]Searles and Armstrong (1970).

[4]Hartman and Dryden (1974).

[5]Thompson et al. (1972).

[6]Dornbush et al. (1940).

[7]Greenhalgh (1970).

put their cows to pasture as there was still snowfall (Meteorological chart, Appendix II). Possibly, farmers could have run out of hay and silage in late winter and used dehydrated alfalfa pellets, as a supplementary forage. Dehydrated hay may contain more β-carotene than the regular winter forages. The observed increase in the vitamin A content of late spring and early summer milk (Figure 11) could have been due to the transfer of cattle from winter feeds to pasture. Fresh spring and summer pastures contain high levels of β-carotene. Kirchgessner et al. (1967) reported mean values of 40-100 mg β-carotene per kg fresh forage which is greater than the β-carotene required to produce average vitamin A levels of milk (Table 11, Figure 11). β-carotene levels were observed to rise slowly in spring and decline slowly in fall in accordance with the observations of Thompson (1968) but in contrast to the reports of Smith (1967) and Searles and Armstrong (1970) who noted a sudden increase and a sudden decline in the β-carotene levels of spring and fall milk, respectively. The β-carotene contribution to the total potency of milk was greater in summer (20.03%) than in winter (14.47%). These values are comparable with values of 23.70% for summer and 13.80% for winter of the Manitoba-Saskatchewan-Alberta pooled milk samples (Searles and Armstrong, 1970).

5.2.2 Effect of supplementaiton of winter forages (silage and hay) with vitamin A on the vitamin A potency of milk

Ten lactating Holstein cows from the University of Saskatchewan dairy herd were fed increased levels of vitamin A (about 10 times the RDA levels) for a period of 10 days. The vitamin A content of the resulting milk (Table 15, Figure 13) rose by 24% within the first three days of supplementation, implying that there was no lag phase in achieving maximal levels of vitamin A in the milk. Upon removal of the extra supplementation, the

vitamin A levels in the milk declined rapidly. The observations that the vitamin A content of milk rises suddenly upon supplementation and declines rapidly upon removal of the supplement are useful especially when designing vitamin A feeding experiments. The milk yield and butter fat content of the milk (Table 18) were not affected by the extra supplementation. Swanson (1968) and Thompson (1975) had also reported that vitamin A is not related to milk yield.

In order to further investigate the effect of vitamin A supplementation on the vitamin A content of milk, a second feeding experiment was conducted. A 4 x 4 Latin square design was used, with each period comprising 28 days. Wheat silage and alfalfa hay were each supplemented with two levels of vitamin A: 250,000 and 600,000 IU/cow/day, as described in Materials and Methods (section 3.4.8). The resutls for vitamin A recoveries in the milk (Table 21A & B) indicate that i) the higher levels of supplementation resulted in higher vitamin A recoveries in the milk than the lower levels of supplementation and ii) fortification of silage with vitamin A resulted in higher recoveries of vitamin A in the milk than corresponding fortification of hay. Dietary vitamin A recoveries of 4.50% and 3.45% in milk were observed for silage and hay, respectively. The low recoveries of dietary vitamin A in the milk of hay-fed cows could not be accounted for and indicate that there may be a factor in hay with prevents the transfer of dietary vitamin A to the milk. A possible factor may be the high β-carotene consumption (5.8 gm/cow/day) by the hay-fed cows (Table 19). This intake was almost twice as much as the β-carotene intake of the silage-fed cows (Table 19) and about 8 times the

NRC (1971) RDA of β-carotene for maintenance and lactation. In addition, Kirchgessner et al. (1967) had reported that supplementation of a high β-carotene diet with 200,000 IU vitamin A/kg feed had no effect on the vitamin A content of the resulting milk. However, the same level of supplementation on a moderate β-carotene diet increased the vitamin A content of the milk by 108%.

The vitamin A concentration in blood samples (Table 23A) was not significantly influenced by vitamin A supplementation of the feeds. A similar observation was reported by Rusoff et al. (1965) after feeding dairy cows 82,530 IU vitamin A/cow/day. Serum vitamin A is quite resistant to changes in the dietary level of vitamin A due to liver reserves of the vitamin (Rodriguez and Irwin, 1972).

Supplementation of silage with vitamin A caused a non-significant decrease on the β-carotene content of milk (Table 22) and blood (Table 23B). Supplementation of hay, however, significantly ($P \leq 0.05$) decreased the β-carotene of the milk (Table 22) and blood (Table 23B). These results confirm earlier reports that high levels of dietary vitamin A significantly interfere with β-carotene absorption and, hence, reduce the β-carotene content of blood (Rusoff et al., 1965) and of milk (Kirchgessner et al., 1967; Thompson, 1975). The efficiency of conversion of β-carotene into vitamin A could not be calculated in this experiment as β-carotene was fed in conjunction with vitamin A. The recoveries of dietary β-carotene as β-carotene itself in milk was only 0.042% in butter fat. Martin et al. (1969) had obtained dietary β-carotene recoveries of 0.035% in butter fat. These values indicate that very little of the dietary β-carotene is transferred, unconverted, to milk.

Rectal "grab" fecal samples of the experimental cows were analysed for vitamin A and β-carotene. Rectal samples were used rather than whole stool samples to minimize oxidative losses of vitamin A and β-carotene upon exposure to the air. The first analyses were unsuccessful perhaps because the samples were freeze-dried. Almendinger and Hinds (1969), however, did not encounter any problems in the analysis of freeze-dried fecal samples. Negligible amounts of dietary vitamin A (Table 20) and dietary β-carotene (Table 19) were found in the feces of the experimental animals (Tables 24A and B). The lack of high fecal losses of the above nutrients could be accountable to the high overall digestibility values of 98.15% and 55.53% for β-carotene and dry matter, respectively. On the contrary, McGillivray (1951) obtained β-carotene losses of 160% in the feces of sheep and hypothesized that there was microbial synthesis of β-carotene in the rumen. Almendinger and Hinds (1969) obtained fecal β-carotene levels ranging from 83.40% to 90.10% of dietary β-carotene from hay and 172% to 184% of dietary β-carotene from silage fed to beef cattle. They postulated that the rigorous methods used in the extraction of fecal β-carotene may release residual carotenoids that are not released by the feed extraction procedures. More recently, Sweeny and Marsh (1974) obtained only 12.40% recoveries of dietary β-carotene in feces of rats and disagreed with Almendinger and Hinds (1969) theory of residual carotenoids.

5.3 Effect of processing, marketing and storage on the vitamin A potency of milk

All commercial milk intended for fluid consumption must undergo pasteurization at 72°C for 15 seconds. The vitamin A and β-carotene contents of commercial milk were analysed monthly for a period of one year,

prior to and after pasteurization. This was to determine whether the heat treatment during pasteurization affected the vitamin A potency of the milk. The results (Table 5) indicate that pasteurization had no significant effect on the vitamin A potency of milk, in agreement with reports of Wanner (1973), Hartman and Dryden (1974) and Owen and McIntire (1975). The vitamin A values of pasteurized milk obtained in this study (Table 5) fall within the range of 119 - 176 IU vitamin A/100 ml, reported by Owen and McIntire (1975). Pasteurized milk had 8.06% more vitamin A and 10.50% less β-carotene than the unpasteurized milk. The increase in vitamin A and the corresponding decrease in β-carotene contents of milk upon pasteurization could be due to oxidation products of β-carotene as hypothesized by Thompson (1968) and Satter et al. (1977).

The process of skimming milk is performed to obtain skim milk (0.05% B.F.) and partially skimmed milk (2% B.F. milk) which are required by people incapable of digesting fat; those who are on weight-reducing diets and for infant formulae. Unfortunately, the skimming process results in the loss of the fat-soluble vitamins, particularly vitamin A. The vitamin A potency of whole and skim milk samples was analysed for a period of one year. The results (Table 6) show that 85% and 26.50% of the vitamin A content of whole milk was lost upon complete and partial (2% B.F.) skimming of milk, respectively. Rakes and Potts (1965) reported a loss of 88.50% of the vitamin A of whole milk upon complete skimming. More recently, Thompson et al. (1972) observed a vitamin A loss of 66.60% in pooled samples of 2% B.F. milk from five Canadian cities.

The vitamin A content of skim milk and 2% B.F. milk can be restored by fortification of the milk with synthetic vitamin A. Commercially

fortified skim milk and 2% B.F. milk with 150 IU retinyl palmitate added per 100 ml were found to contain 14.55 and 2.36 times more vitamin A, respectively, than the corresponding unfortified samples. Thompson et al. (1972) reported that fortified 2% B.F. milk had 2.25 times more vitamin A than a corresponding unfortified sample. Fortified 2% B.F. milk appears to be a better source of vitamin A than pasteurized 3.25% B.F. milk (Figure 10). However, three of the twelve samples of fortified 2% B.F. milk were discovered to contain about 35% less vitamin A than the expected level of 150 IU/vitamin A added per 100 ml. This emphasizes the need for efficient fortification of low fat milk.

Milk and milk products are displayed under fluorescent light in food stores for prolonged hours; resulting in a potential loss of vitamin A and β-carotene due to photodegradation. Light cannot be excluded from food stores for marketing and economic reasons. The photodegradation of vitamins in foods must, therefore, be reduced through proper packaging materials which can screen out damaging wavelengths. Although vitamin A was found to be destroyed by light in both the ultraviolet and visible spectra, maximal photodegradation occurred between 340 to 440 nm (Table 3). Satter et al. (1977) reported that maximal photodegradation of vitamin A and β-carotene in solutions of milk fat occurred below 415 and 465 nm, respectively. Photodegradation of vitamin A was observed to increase with exposure time (Table 3). Minimization of vitamin A loss by photodegradation can be achieved either by use of a proper packaging material or reduction of the exposure time. In addition, β-carotene protected against photodegradation of vitamin A (Table 4). Satter et al. (1977) had reported similar protective effects of β-carotene. β-carotene or its photodegradation

products may absorb the radiation that would otherwise degrade vitamin A.

Refrigerated commercial fluid milk usually has a shelf-life of two to three weeks before the fat deteriorates. Storage experiments (Table 7) indicated that the lower the storage temperature, the greater the stability of the vitamin A in the milk. Whole milk was observed to undergo less vitamin A loss than either skim, 2% B.F. or homogenized (3.25% B.F.) milk. Similar observations were made by Rakes and Potts (1965) and more recently by Bauernfeind and Cort (1974). The high fat content of whole milk may protect the vitamin from oxidation by preferential oxidation. Such protective effects were not observed in homogenized (3.25% B.F.) milk which may be due to the fat globules being broken down. Artificially added vitamin A is lost from milk at a greater rate than the vitamin that is naturally present (Table 7). Thompson and Erdody (1972) observed that the vitamin A of fortified 3.25% homogenized milk underwent more rapid degradation than did the vitamin A of unfortified samples. They concluded that synthetic vitamin A is unstable. These results emphasize the need for proper packaging, storage and handling of processed milk in food stores and in homes.

6. SUMMARY AND CONCLUSIONS

The factors affecting the vitamin A and β-carotene contents of milk were investigated. A series of short-term and long-term experiments were conducted, using the University of Saskatchewan dairy herd and Saskatoon commercial fluid milk, respectively.

The milk of cows in late lactation contained more vitamin A per 100 ml but the milk of cows in early lactation contained more vitamin A per gm butter fat. Cow-to-cow and day-to-day variations in the vitamin A content of milk were significant under defined conditions of dietary vitamin A and stages of lactation. Morning milk had more vitamin A per gm butter fat while afternoon milk had more vitamin A per 100 ml.

Pronounced seasonal variations were observed in Saskatoon market milks. Spring and summer milk had significantly more (1.17 times) vitamin A than that of winter and fall milk. Supplementation of winter forages with vitamin A increased the vitamin A in milk. Dietary hay suppressed the recovery of dietary vitamin A in milk. High levels of dietary vitamin A depressed the β-carotene content of milk and blood. The concentration of vitamin A in blood was not affected by dietary levels of the vitamin. No significant amounts of dietary vitamin A and β-carotene were detected in the feces of the experimental animals.

Pasteurization was found to have no significant effect on the vitamin A and β-carotene contents of milk. The process of skimming significantly reduced the vitamin A content of the resulting skim and 2% B.F. milk. Fortification significantly increased the vitamin A content of milk above

the levels of the vitamin that are naturally present in milk. Maximal vitamin A photodegradation was observed to occur between 340 to 440 nm. β-carotene significantly reduced the extent of vitamin A photodegradation. Loss of vitamin A in stored milk was observed to be accelerated by high storage temperatures, prolonged storage periods and processing of the milk.

In conclusion, the vitamin A potency of milk can be optimized by fortification of winter feeds, especially silage, with high levels of vitamin A. After the milk has been produced, the vitamin A can be maintained by proper fortification of low fat milk and by protection of processed milk from deleterious wavelengths using proper packaging and storage conditions.

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APPENDICES

Appendix I

Calculation formulae for vitamin A and β-carotene

A. Vitamin A content in milk

$$[\text{Vitamin A}] = \frac{\text{Absorbance-Intercept}}{\text{slope}} \times \frac{\text{mg vitamin A per ml}}{\text{of working standard}} \times \frac{1000}{.344} \times 200 \times 2$$

$$= \text{IU/100 ml}$$

Divide the results of the above calculation by % B.F. to get vitamin A content expressed as IU/gm B.F.

B. β-carotene content of milk

$$[\beta\text{-carotene}] = \frac{\text{Absorbance-Intercept}}{\text{slope}} \times \frac{\text{mg β-carotene per}}{\text{ml working standard}} \times \frac{1000}{.6} \times 200 \times 2$$

$$= \text{IU/100 ml}$$

Divide the results of the above calculation by % B.F. to get β-carotene content expressed as IU/gm B.F.

C. Vitamin A content of concentrate feed

$$[\text{Vitamin A}] = \frac{\text{Absorbance-Intercept}}{\text{slope}} \times \frac{\text{mg vitamin A per}}{\text{ml working standard}} \times \frac{200}{\text{weight of sample}} \times \frac{1000}{.344} \times 1000$$

$$= \text{IU/kg feed}$$

Appendix I continued

D. Vitamin A concentration in blood samples

$$\text{Vitamin A} = \frac{\text{Absorbance-Intercept}}{\text{slope}} \times \frac{\text{mg vitamin A per ml}}{\text{of working standard}} \times \frac{\text{ml petroleum ether added initially}}{\text{ml petroleum ether in aliquot}} \times \frac{100}{\text{ml serum used}} \times \frac{1000}{.344}$$

$$= \text{IU/100 ml}$$

E. β-carotene concentration in blood samples

$$\beta\text{-carotene} = \frac{\text{Absorbance-Intercept}}{\text{slope}} \times \frac{\text{mg β-carotene per ml}}{\text{of working standard}} \times \frac{100}{\text{ml serum used}} \times \frac{1000}{.6}$$

$$= \text{IU/100 ml}$$

F. Vitamin A content of fecal samples

$$\frac{\text{IU vitamin A per}}{\text{100 gm feces}} = \frac{\text{Absorbance-Intercept}}{\text{slope}} \times \frac{\text{mg vitamin A per ml}}{\text{working standard}} \times \frac{\text{final volume of digesta (100 gm)}}{\text{ml of digesta extracted (10 ml)}} \times \frac{\text{final volume of petroleum ether extract (50 ml)}}{\text{weight of sample (50 g)}} \times \frac{1}{\text{ml of petroleum ether extract evaporated (5 ml)}} \times \frac{1000}{.344} \times 2$$

Appendix I continued

G. β-carotene content of fecal samples

$$\text{IU β-carotene per 100 gm feces} = \frac{\text{Absorbance-Intercept}}{\text{slope}} \times \text{mg β-carotene per ml working standard} \times \frac{\text{final volume of digesta}}{\text{ml of digesta extracted}} \times \frac{\text{final volume of petroleum ether extract}}{\text{weight of sample}} \times \frac{1}{\text{ml of petroleum ether extract evaporated}} \times \frac{1000}{.6} \times 2$$

H. β-carotene content of dried forages

$$\text{mg β-carotene per kg forage material} = \frac{\text{Absorbance-Intercept}}{\text{slope}} \times \text{mg β-carotene per ml working standard} \times \frac{200}{\text{weight of sample}} \times 1000$$

Appendix II

Mean[1] Precipitation for Saskatoon Region (June 1977 - July 1978)

Month of the year	Rainfall (mm)	Snowfall (cm)
June 1977	65.5	-[2]
July	33.8	-
August	27.8	-
September	47.1	-
October	0.7	4.3
November	4.0	7.7
December	0.4	29.9
January 1978	-	12.3
February	1.2	4.0
March	2.1	2.6
April	17.9	14.1
May	34.3	-
June	39.9	-
July	56.0	-

[1]Mean of 30 days.

[2]Indicates no rain or snowfall in the whole month.

Source: Saskatoon Weather Office