Fall 2012

Variations in Carotenoids in Plasma From Jersey Cows at an Organic Dairy Compared to a Conventional Dairy Over Time

Jillian M. Smith
University of New Hampshire - Main Campus

VARIATIONS IN CAROTENOIDS IN PLASMA FROM JERSRY COWS AT AN ORGANIC DIARY COMPARED TO A CONVENTIONAL DAIRY OVER TIME

BY

JILLIAN M. SMITH
University of New Hampshire

THESIS

Submitted to the University of New Hampshire in
partial fulfillment of the requirements for a
Bachelor of Sciences degree in Nutrition under the
Honors in Major Program.

December, 2012

Table of Contents

Abstract

Interest in enhanced health promoting properties of foods, as well as a concern over the environmental and social impact of food sourcing are challenging the conventional food system. This is evidenced in the small but steady demand for organic milk. By USDA Certified Organic standards, animals must be fed on pasture for a minimum of 120 days in order for their products to be classified as organic. Research indicates that various factors including diet, breed, and stage of lactation all impact the nutritional value and composition of milk. The objective of this study was to understand how feeding practices affected bovine plasma carotenoid concentration over time, and how these changes related to changes in the bovine milk carotenoid concentration over time. Plasma samples were collected from 9 Jersey cows fed on pasture at an organic dairy and from 9 Jersey cows fed total mixed ration (TMR) at a conventional dairy. Components of interest were extracted from the plasma, then separated and quantified by High Performance Liquid Chromatography. Data were analyzed using Microsoft® Office Excel 2007 (Microsoft Corporation, Redmond, WA) and SPSS® version 19.0 (SPSS Inc. Chicago, IL). Results showed that plasma concentrations of lutein, zeaxanthin, β-cryptoxanthin, and β-carotene significantly changed from baseline at each dairy. The changes in plasma lutein, zeaxanthin, α-carotene, and β-carotene concentrations were significantly different between dairies. Plasma lutein, zeaxanthin, α-carotene, and β-carotene concentrations were also significantly higher in plasma from cows that consumed pasture versus cows fed TMR. Additionally, plasma carotenoid concentration was greater than milk carotenoid concentration over the course of the study. Changes in plasma and milk carotenoid concentrations occurred in similar patterns over time suggesting that the plasma carotenoid concentration is a good indicator of the milk carotenoid concentration.

Introduction

Carotenoids

Phytonutrients, "phyto" originating from Greek word for plant, are organic components of plants that are thought to be beneficial for human health. Common sources of these components are fruits, vegetables, legumes, grains and nuts. There are many classes of phytonutrients each with different chemical compositions and functions. They are classified as carotenoids, phenolics, alkaloids, N-containing compounds, and organosulfur compounds. While the functions of these chemicals within the plant are generally understood, research continues to investigate the physiological effects of phytonutrients within the body. Among the most studied phytonutrients are carotenoids.

Carotenoids consist of over 700 fat-soluble compounds that are responsible for the bright colors of foods like carrots, tomatoes, and other dark green, yellow, orange and red fruits and vegetables (1). Unlike photosynthetic bacteria that can biosynthesize carotenoids, animals and humans cannot synthesize these compounds and must consume them in their diet. Although carotenoids are not essential for life, they have been shown to promote human health. Studies have demonstrated that increased consumption of carotenoids may be linked to the decreased risk of cardiovascular disease, cancer, age-related macular degeneration, cataracts, and immune system decline (2, 3).

Carotenoids belong to a group of compounds referred to as tetraterpenoids. A tetraterpenoid is a 40-carbon polyene chain consisting of 4 terpene units each with 10 carbon atoms (1). The long series of carbon atoms have alternating single and double bonds resulting in the delocalization of π-electrons over the length of the chain (1). The polyene

chains may or may not also contain oxygen. These features all combine to give carotenoids their distinct shape, chemical reactivity, and light-absorbing properties (1).

Carotenoids are divided into two classes based on their chemical structures: carotenes and xanthophylls (**Figure 1**). Carotenes are hydrocarbons that contain only hydrogen and carbon atoms. Examples of this class include α-carotene, β-carotene, and lycopene. Xanthophylls are oxygenated derivatives of hydrocarbons. Lutein, zeaxanthin, and β-cryptoxanthin are all classified as xanthophylls. Carotenoids can also be separated by provitamin A and non-provitamin A activity. Due to the presence of at least one non-substituted beta-ionone moiety, specific carotenoids, α-carotene, β-carotene, and β-cryptoxanthin are able to convert into retinol, a precursor of vitamin A (4).

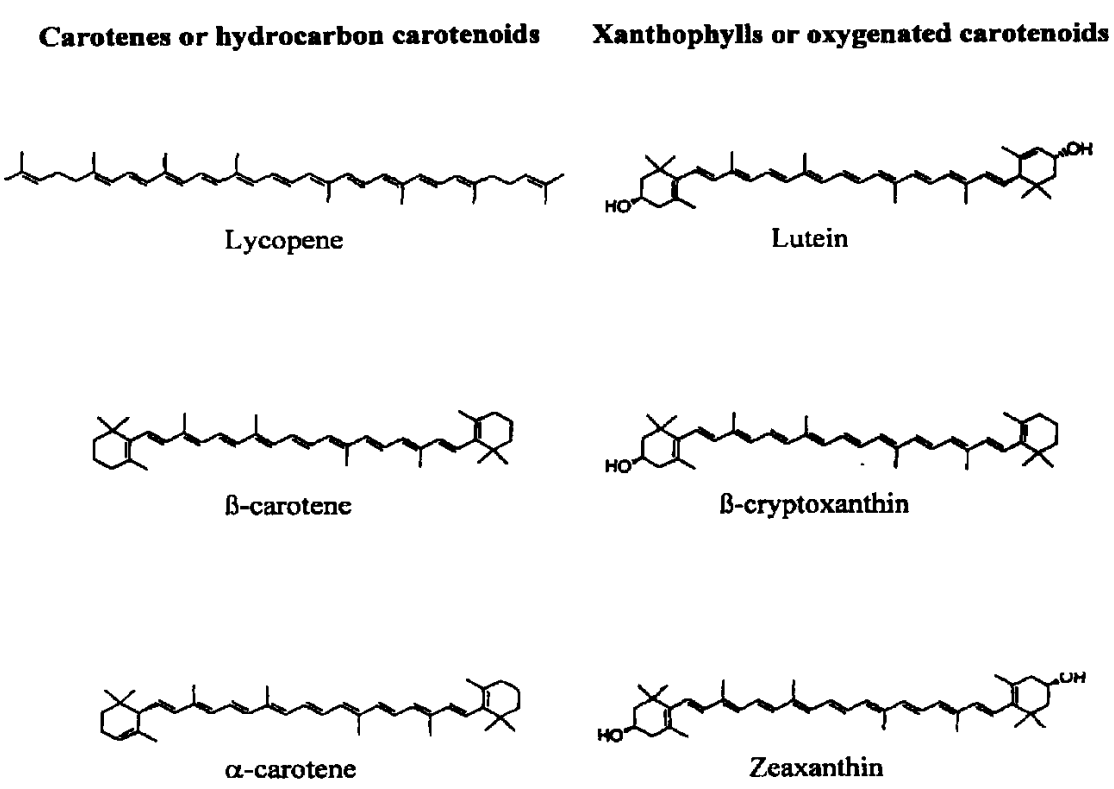


Figure 1. Structures of carotenes and xanthophylls (5)

Functions of Carotenoids

Carotenoids participate in various aspects of photosynthesis. Their chemical properties enable them to absorb light at wavelengths that chlorophyll cannot. When carotenoids become excited, the captured energy is transferred to the reaction centers of photosynthesis (4). The collected energy can then be converted into useful fuel sources for the plant. Carotenoids also help to absorb excess light energy. Plants are periodically exposed to intense sunlight. Because only a small portion of the energy is needed for the production of fuel, much of this energy is left over. The energy overload could potentially damage the plant, but the carotenoids accept the energy reducing the risk of adverse effects (4).

It is proposed that carotenoids also function as antioxidants in the body sequestering free radicals. Free radicals are molecules that contain highly reactive and unstable unpaired electrons (6). Free radicals have the tendency to take electrons from neighboring molecules producing new free radicals in a chain-like pattern. This causes oxidative damage to DNA, cell membranes, blood lipids, and many proteins (6). Many disease are linked to oxidative damage such as cancer, cardiovascular disease, age-related macular degeneration, and cataracts (2, 3).

Free radicals are naturally produced in the body as byproducts of metabolism, but are shown to increase due to pollutant, smoking, drugs, UV rays, and radiation and accumulate with age. Antioxidants act as guards against free radicals, sacrificing themselves in order to protect other molecules from oxidative damage. There are endogenous antioxidant systems in the body. However with environmental stresses and age, these systems could potentially

reach a limit. Adequate consumption of antioxidants, especially carotenoids, may help protect against oxidative stress and the development of several chronic diseases (6).

Some carotenoids, such as α-carotene, β-carotene, and β-cryptoxanthin, also function as precursors to vitamin A. These compounds can be converted to vitamin A when needed by body reducing the potential for vitamin A deficiency (7). The chemical compositions of α-carotene, β-carotene, and β-cryptoxanthin allow them to be bioconverted to retinol mainly through cleavage of C-15, 15' double bond (**Figure 2**) (8). Vitamin A plays a major role in vision, growth and development, reproduction, and immune system function (7).

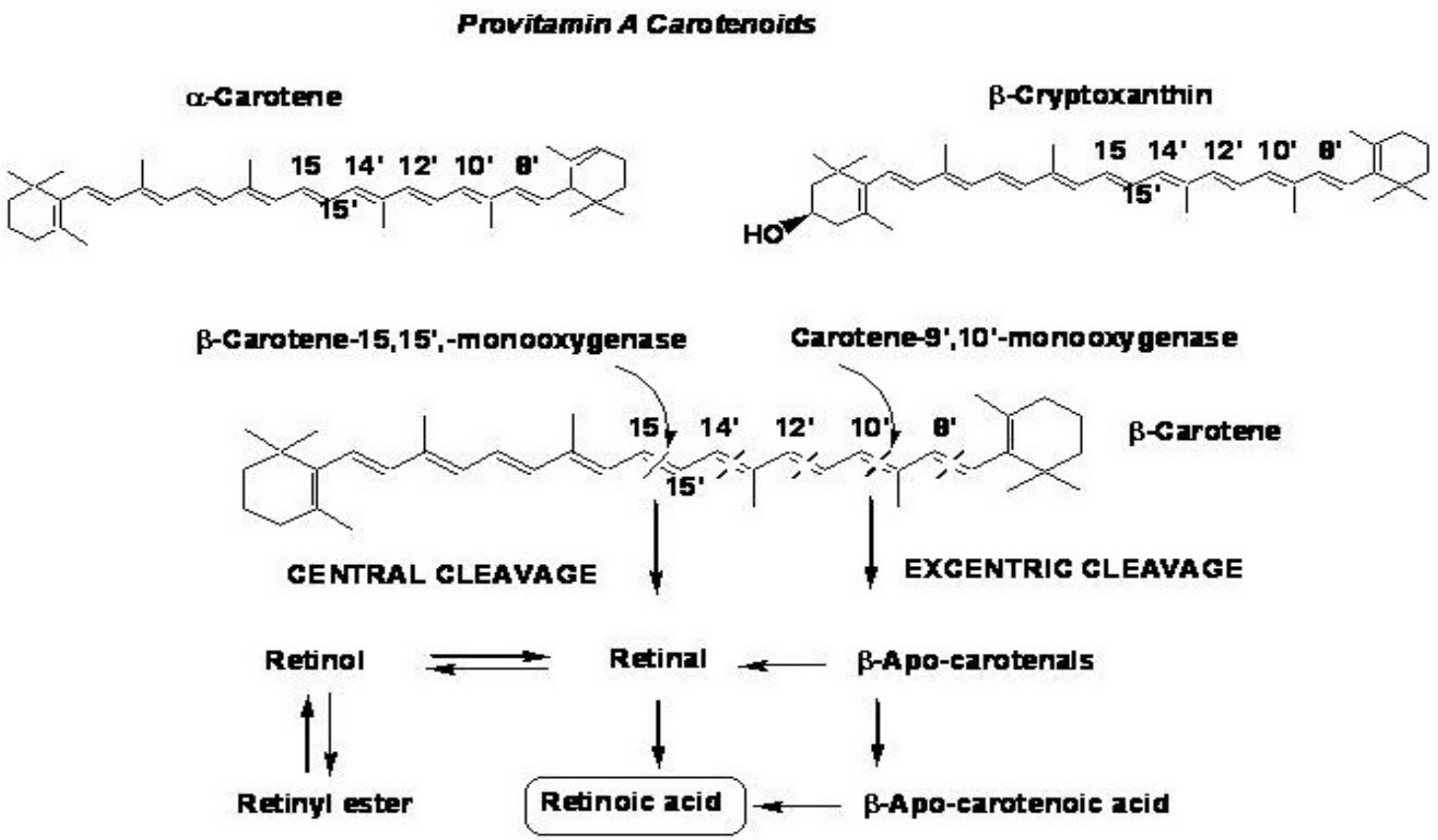


Figure 2. Bioconversion of β-carotene into retinol (a form of vitamin A) (9).

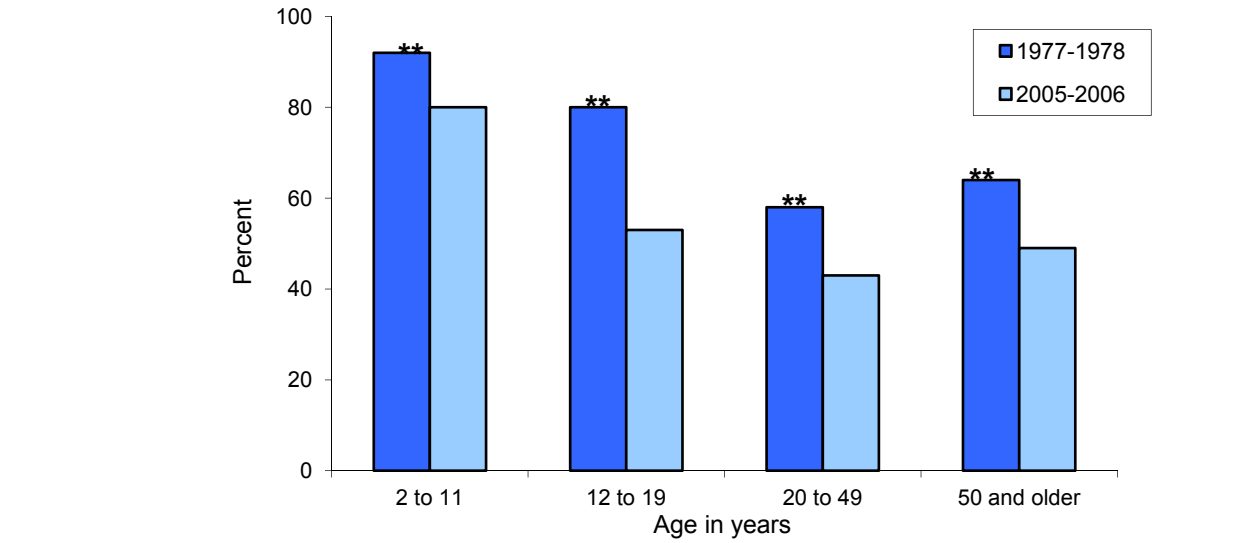
Carotenoids in Milk

A growing segment of the population is becoming more invested in the health promoting or sensory qualities of food products (10). Many individuals want to consume foods that provide added health benefits. Interest in enhancing the nutritional contributions of food is long standing and can be seen throughout the food industry. One particular example is cow's milk and milk products.

Dairy products are widely consumed commodities throughout the lifecycle in the United States. Several instances, such as the use of recombinant bovine growth hormone (rbGH) in milk, have shown that dairy consumers do care about the quality and composition of market products (11). Over the years, the dairy industry has increased fortification of nutrients in milk due to consumer demand. For example, milk products today are typically fortified with both vitamin A and vitamin D (12). This is advantageous because evidence suggests that adequate dairy intake is related to improved bone growth and maintenance (12). According to work summarized by the USDA, milk consumption is also linked to the reduced risk of cardiovascular disease, hypertension, and type II diabetes in the adult population (12).

However, it is clear that fluid milk consumption has decreased in the United States. In 1977-1978, 76% of adolescents reported milk consumption while in 2005-2006 only 48% of adolescents reported milk consumption (**Figure 3**) (12). One way to regain interest in milk and milk products is to reevaluate their nutrient contents and to increase the density of

health promoting nutrients. A possible way to add value to the nutrient composition of milk is to increase the concentration of carotenoids.



NOTES: ** Statistically significant difference between survey years (p<.01).
SOURCES: Nationwide Food Consumption Survey 1977-1978 and What We Eat in America, NHANES 2005-2006, Day 1 dietary intake data, weighted.

Figure 3. Percent of individuals consuming fluid milk by age group, 1977-1978 and 2005-2006 (12).

The most abundant carotenoid in cow's milk is β-carotene. However trace amounts of lutein and other carotenoids have also been identified in cow's milk, in addition to α-tocopherol and retinol (13). The concentration of carotenoids in cow's milk is dependent on many factors including breed, stage of lactation, age, seasonal variations, and farming practices. These factors must all be considered when studying the composition of the milk. As with many of the nutrients, carotenoid concentration in cow's milk varies with carotenoid concentration in the diet. Evidence shows that high consumption of carotenoids via the food supply leads to higher concentration of carotenoids in the milk. Noziere et. al. studied this relationship contrasting hay silage diets with grass silage diets. Due to the higher concentration of carotenoids in the grass silage diet, cows consuming this forage

produced milk with higher carotenoid concentrations in relation to cows consuming the hay silage diet (13). Feeding strategies have also been shown to have an effect on the milk carotenoid concentration (14). Pasture feeding has a long-term association with higher carotenoid concentration in the milk (15). This is primarily due to the natural consumption of green grasses as the main food source for the cow. The carotenoid concentration of green grasses is greater than those of hay and conventional corn silages, which can be illustrated in the carotenoid concentration of the respective cow's milk (16).

In addition, seasonal variations have been shown to alter the concentration of carotenoids in cow's milk. This is primarily due to the nature of the forage provided over time (17). During periods when grasses are plentiful and used as the major food source for cows, the carotenoid concentration of the cows' milk tends to increase (17). However, when grasses are scarce or unavailable, dietary supplementation of hay and corn forages decreases the milk carotenoid concentration (17). In geographic areas in which seasonal variability is limited, the changes in grass type and composition have also been shown to effect the milk carotenoid concentration as well (18).

Carotenoids in Plasma

It is important to study the plasma carotenoid concentration to evaluate the relationship between dietary consumption and the carotenoid concentrations in milk. The blood is the main source of the chemical composition of milk and thus carotenoids. Since there is limited biosynthesis of carotenoids in mammals, carotenoids consumed in the diet are digested and then absorbed into the blood stream. Understanding the relationship between

the diet composition and the carotenoids transferred from the plasma to the milk is beneficial for both the consumer and the farmer.

It is known that a relationship does exist between the carotenoids in plasma and the carotenoids in milk. However, carotenoid conversion from the plasma to the milk is not absolute. Previous studies have illustrated the association between the carotenoid concentrations of plasma and milk. One study showed the plasma samples consisted of both *13-cis-β-carotene* and *all-trans-β-carotene* while the milk samples only contained all-*trans*-β-carotene (4). The same study illustrated that 1 to 3% of the blood plasma carotenoids were attributed to lutein while only trace amounts of lutein were measured in milk (4). Other studies have reported different results. For example, Calderon et al. found that lutein accounted for up to 18% of the total carotenoids in cow's milk (8). Therefore the relationship of dietary to plasma to milk carotenoids is inconclusive. With interest in organic farming rising, a practice that requires animals to be fed on pasture for a minimum of 120 days, it is critical to examine this association to appreciate the variability of milk composition over time.

The objective of this study was to understand the relationship between the carotenoid concentration of the cow's plasma and the carotenoid concentration of the cow's milk. The study examined the nature of this relationship and how it changed over time in Jersey cows at a conventional dairy farm and an organic dairy farm. Evaluating the plasma carotenoids of these cows allowed inferences to be made about the impact of the cow's dietary intake on the nutrition quality of the milk by exploring the transferability of dietary carotenoids from the food supply, to the plasma, and ultimately to the milk.

Objectives

1. To observe the changes in the plasma carotenoid concentration over time and season
2. To observe how two feed practices at the two University of New Hampshire Dairy Research Facilities effect the plasma carotenoid concentration
3. To understand the relationship between the plasma carotenoid concentration and the milk carotenoid concentration of the studied Jersey cows at the two University of New Hampshire Dairy Research Facilities

Hypotheses

1. Plasma carotenoids from Jersey cows at the Organic Dairy Research Facility and the Fairchild Dairy Teaching and Research Center will differ significantly by the second month of pasture and will continue to differ significantly through the end of the pasture feeding
2. The plasma carotenoid concentration will be higher in the cows from the Organic Dairy Research Facility (pasture) than the cows from the Fairchild Dairy Teaching and Research Center (TMR)
3. The trends in the plasma carotenoid concentration over time will be comparable to the changes in the milk carotenoid concentration over time, but the changes will be syncopated (i.e. the plasma carotenoid concentration will increase before the milk carotenoid concentration)
4. Some carotenoids that are present in the plasma will not be present in the milk

Materials and Methods

Collection of Plasma Samples

Blood samples were collected every two weeks at approximately 1600hrs from nine Jersey cows at the UNH Organic Dairy Research Facility and nine Jersey cows at the UNH Fairchild Dairy Teaching and Research Center. Collections began May 11, 2011 and continued through to November 10, 2011. Blood was extracted from the coccygeal (tail) vein or artery using 1", 21 gauge needles (Kendall Monoject) and put into 10mL Vacutainer tubes with K_2 EDTA. Plasma samples were then transferred into 2mL Microtubes in aliquots of three and store at -80^{0}C until needed for analysis.

Carotenoid Extractions

Plasma carotenoid extractions were completed on one sample set per month in duplicates. Samples were retrieved from the -80^{0}C freezer and the cow number, date, and dairy facility were recorded in a Microsoft® Office Excel 2007 (Microsoft Corporation, Redmond, WA) spreadsheet. To measure the carotenoid concentrations in the plasma samples, the standard laboratory method based on the Handelman et al. carotenoid extraction process was used (19). Due to the high concentration of carotenoids in the plasma samples, the samples were diluted for the extraction process to ensure the concentrations were within the range previously set for the High Performance Liquid Chromatography (HPLC) system.

Frozen plasma samples were thawed in a warm water bath at approximately 30^{0}C. Once thawed, 50µL of plasma were pipetted into 16 x 100 culture tubes (Fisher #14-961-29) using a glass Pasteur pipette. 100uL of internal standard (ISTD) and 100µL of ethanol

(Acros 200 proof, Fisher #61509-0020) were then added to each sample. The internal standard was ethyl-8'-apo-β-caroten-8'-oate (Carotenature, Lupsingen, Switzerland). The samples were vortexed lightly and then 2mL of hexane (Optima, Fisher #H303-4) were added to the samples. The samples were capped, vortexed for 45 seconds, and then centrifuged for one minute at 100 x g at 20^0C. Once centrifuged, the upper organic layer was removed from each sample using a pipette and added to individual clean 16 x 100 culture tubes. 2mL of hexane were then added to each original culture tube consisting of precipitated material. The samples were capped, vortexed for 45 seconds, and centrifuged for one minute at 100 x g at 20^0C. The top organic layer was removed again and added to the respective culture tubes with the organic layer from the first extraction cycles. The culture tubes containing the organic layers from the two extractions were dried down using a nitrogen evaporator. When completely dry, the samples were rehydrated with 100μL ethanol and vortexed for one minute. The contents were then transferred to amber HPLC vials (11mm, 2.0 mL, Restek, Fisher #24385) with glass inserts (200μL flat bottom – Restek, Fisher #24385) and crimped sealed (11mm PTFE, Restek, Fisher #14-930-15E) for High Performance Liquid Chromatography (HPLC) analysis.

High Performance Liquid Chromatography Analysis

Components extracted from the plasma were lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, and 13-*cis* β-carotene, retinol and α-tocopherol. The carotenoids were the main focus of the study, but retinol and α-tocopherol were included in the analysis as the data may be beneficial for further research. To analyze the concentration of the carotenoids, retinol, and α-tocopherol in plasma a Hewlett Packard/Agilent Technologies 1100 series High Performance Liquid Chromatography (HPLC) system with a photodiode

array detector (Agilent Technologies, Palo Alto, CA) was used (20). The different analytes were separated based on size, solubility, and charge using a 5μm, 200 Å polymeric C30 reverse-phase column (Pronto-SIL, MAC-MOD Analytical Inc., Chadds Ford, PA) with a 20μL injection series (20). Two different mobile phases were used to elute the analytes from the column. The HPLC mobile phase organic solvent A was made up of methanol/*tert*-butyl methyl ether/water (83:15:2, vol/vol/vol, with 1.5% ammonium acetate in the water). The second organic solvent, organic solvent B, was made up of methanol/*tert*-butyl methyl ether/water (8:90:2, vol/vol/vol, with 1% ammonium acetate in the water).

The gradient method of HPLC at a flow rate of 1mL/min began at 100% Solvent A for 2 minutes. Over a 6 minute linear gradient, Solvent A was reduced to 70% and held at 70% Solvent A for 3 minutes. Then on a 10 minute linear gradient, Solvent A was reduced to 5% and held at 5% solvent A for 4 minutes, and finally, increased backed to 100% Solvent A on a 2 minute linear gradient. Once at 100% Solvent A, the system was held for 10 minutes to restore initial conditions.

Carotenoids were detected at 452nm, retinol was detected at 325nm, and α-tocopherol was detected at 290nm and identified by comparing the spectral analysis and elusion times to those of pure standards (>95%). Concentrations of the different compounds were calculated by using a standard curve and adjusted by the percentage recovery of the internal standard. The mean carotenoid concentration for each sample in each time period was computed. The mean was then adjusted to reflect the concentrations of components in the original sample.

Statistical Analysis

Data collected throughout the extraction period were entered in Microsoft® Office Excel 2007 (Microsoft Corporation, Redmond, WA). For each time point, the mean concentration of each measurement was calculated. The means were then adjusted to reflect the average concentration in the original sample. The sample table below depicts a compilation of the adjusted means (**Table 1**). The "group" column was used to designate the dairy facility the cows resided at. The numbers in the "week" column were assigned based on the collection date. The "code" specified the different cow subjects. The means for each component are in the subsequent respective columns. This data were also used to generate figures to illustrate trends in the data.

Table 1. Adjusted means of components for each cow at both dairy facilities for month three of data collection

				Retinol	**A-toc**	**Lutein**	**Zeaxanthin**	**B-crypto**	**A-Caro**	**B-Caro**	**13c B-Caro**
#	Group	Week	Code	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
1	1	3	14	0.3694	6.56	1.0552	0.0352	0.1214	1.10172	9.775	0.10196
2	1	3	18	0.395	5.839	0.2713	0.03188	0.14961	1.1376	10.939	0.13022
3	1	3	23	0.3166	6.684	0.3517	0.05935	0.12394	0.9546	9.364	0.10791
4	1	3	24	0.2914	7.545	0.2753	0.04361	0.13646	1.4614	13.274	0.18102
5	1	3	26	0.3102	5.827	0.2448	0.04372	0.08791	0.6019	6.512	0.03867
6	1	3	36	0.3922	3.86	0.2256	0.05126	0.15137	1.0792	7.51	0.45199
7	1	3	43	0.4743	6.049	0.2747	0.04151	0.12216	0.8874	9.71	0.45642
8	1	3	46	0.3571	4.123	0.2258	0.0488	0.12774	0.8866	7.584	0.17647
9	1	2	1059	0.2958	6.048	0.16825	0.0323	0.11846	1.1351	10.317	0.13254
10	2	3	82	0.3447	8.636	0.06871	0.016802	0.13488	0.416	3.437	0.017224
11	2	3	2210	0.4112	7.101	0.05391	0.012941	0.109	0.2919	2.689	0
12	2	3	2086	0.4248	7.658	0.07651	0.012694	0.15659	0.4011	3.515	0.007838
13	2	3	2371								
14	2	3	2356	0.327	8.401	0.09147	0.009125	0.17835	0.4018	3.434	0.00698896
15	2	3	2378	0.4644	9.963	0.10859	0.0271	0.2298	0.5647	4.721	0.03973
16	2	3	2101	0.2921	2.527	0.03252	0	0.05807	0.2128	1.7096	0.0005859
17	2	3	2209	0.3616	5.445	0.04997	0.01229	0.11073	0.2906	2.547	0.004747
18	2	3	2177	0.3243	8.493	0.0892	0.018315	0.1827	0.3933	3.313	0.010581

To better demonstrate the trends over time and between the two dairy facilities, the average carotenoid concentrations were calculated for each dairy at each time point. In other words, the adjusted mean concentrations for the individual cows at each dairy for each time point were averaged together. These calculated values were put into tables representing the two dairies (**Table 2**). This data were used to generate figures to better visualize the trends over time.

Table 2. Adjusted means of each component from May to November at the organic dairy

OD	Retinol	A-toc	Lutein	Zeaxanthin	B-crypto	A-Caro	B-Caro	13c B-Caro
1	0.3023	7.038	0.1899	0.02942	0.1286	0.951	9.954	0.055053
2	0.299	5.526	0.1691	0.023931	0.1628	1.107	10.37	0.10101
3	0.356	5.84	0.3436	0.04307	0.1266	1.0273	9.443	0.1974667
4	0.3229	9.205	0.2436	0.04133	0.0837	0.8915	8.403	0.10586
5	0.375	7.3226	0.269	0.05407	0.1292	1.0117	10.32	0.11831
6	0.391	7.81	0.2681	0.04356	0.1483	1.232	12.36	0.0006
7	0.324	4.53	0.1879	0.01589	0.1304	1.101	8.177	0.168307

SPSS® version 19.0 (SPSS Inc. Chicago, IL) was used to further analyze the data. To compare baseline plasma carotenoid concentrations between the UNH Organic Dairy Research Facility and the UNH Fairchild Dairy Teaching and Research Center independent-samples t-tests were performed. A repeated-measures Analysis of Variance (ANOVA) was used to measure changes in plasma carotenoid concentrations over time ($p < 0.05$). The same repeated-measures ANOVA was also used to measure changes in plasma carotenoid concentrations between dairies and over time evaluating the interactions between group and time ($p < 0.05$). Due to lack of data, 13-*cis* β-carotene was not used for this analysis.

Results

The most abundant carotenoid found in the cows' plasma was β-carotene, however lesser amounts of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and 13-*cis* β-carotene were also detected. Retinol and α-tocopherol were identified in the plasma as well. The mean baseline plasma carotenoid concentration is presented below for both the UNH Organic Dairy Research Facility and the UNH Fairchild Dairy Teaching and Research Center (**Table 3)**. At baseline, there was no significant difference in plasma retinol, α-tocopherol, lutein, zeaxanthin, β-cryptoxanthin, and α-carotene concentrations between the two dairies. Plasma β-carotene and 13-*cis* β-carotene concentrations were significantly different at baseline between dairies ($p<0.001$). As evidenced, β-carotene (0.951mg/dL ± 0.35) and 13-*cis* β-carotene (9.954mg/dL ± 3.52) concentrations are both higher at the organic dairy and the conventional dairy (0.381mg/dL ± 0.07) and (3.175mg/dL ± 0.71) respectively.

Table 3. Mean plasma retinol, α-tocopherol, and carotenoid concentrations for the UNH Fairchild Dairy Teaching and Research Center and the UNH Organic Dairy Research Facility at baseline

	UNH Organic Dairy Research Facility		UNH Fairchild Dairy Teaching and Research Center			
Nutrient	**Mean**	**±SD**	**Mean**	**±SD**	**t**	**p**
Retinol	0.302[1]	±0.09	0.362	±0.04	1.82	0.08
α-tocopherol	7.038	±1.89	6.718	±1.30	0.41	0.68
Lutein	0.190	±0.07	0.107	±0.09	2.03	0.06
Zeaxanthin	0.029	±0.01	0.023	±0.02	0.84	0.41
β-cryptoxanthin	0.219	±0.08	0.130	±0.11	1.85	0.08
α-carotene	0.129	±0.04	0.138	±0.04	0.45	0.65
β-carotene	0.951	±0.35	0.381	±0.07	4.77	<0.001*
13-*cis* β-carotene	9.954	±3.52	3.175	±0.71	5.67	<0.001*

[1]Plasma concentrations are expresses in mg/dL.
*Significance ($p<0.05$)
t-value and p-value were predicted on an independent samples t-test

Over time, significant changes from baseline ($p<0.05$) in certain plasma carotenoid concentrations, as well as α-tocopherol, occurred at both dairies (**Table 4)**. This indicates that various factors over the seven-month period led to an increase or a decrease in the components of interest. At the organic dairy, lutein and zeaxanthin varied significantly from baseline throughout the study, ($F = 15.427$, $p < 0.001$) and ($F = 11.282$, $p <0.001$) respectively. Plasma concentrations of β-cryptoxanthin and β-carotene also changed significantly from baseline, ($F = 5.178$, $p = 0.001$) and ($F = 4.172$, $p = 0.004$) respectively. A significant change from baseline was also observed for α-tocopherol ($F= 4.112$, $p = 0.003$). Plasma concentrations of retinol, α-carotene, and 13-*cis* β-carotene did not significantly change from baseline over time at the organic dairy or the conventional dairy.

Table 4. Changes over tine and season in retinol, α-tocopherol, and carotenoid concentrations for the UNH Fairchild Dairy Teaching and Research Center and the UNH Organic Dairy Research Facility from baseline

	Changes from baseline	
Nutrient	**F**	**p**
Retinol	2.101	0.073
α-tocopherol	4.112	0.003
Lutein	15.427	<0.001
Zeaxanthin	11.282	<0.001
β-cryptoxanthin	5.178	0.001
α-carotene	1.117	0.372
β-carotene	4.172	0.004
13-*cis* β-carotene	1.521	0.228

F-value and P-value were calculated by two-way repeated measures ANOVA

The interactions between group (UNH Organic Dairy Research Facility or UNH Fairchild Dairy Teaching and Research Center) and time (month, May to November) illustrate that plasma lutein, zeaxanthin, α-carotene, β-carotene, and α-tocopherol concentrations vary over time from baseline in significantly different ways at each dairy ($p <0.05$) **(Table 5)**.

Table 5. Changes over tine and season in retinol, α-tocopherol, and carotenoid concentrations between the UNH Fairchild Dairy Teaching and Research Center and the UNH Organic Dairy Research Facility

	Interaction[1]	
Nutrient	**F**	**p**
Retinol	1.48	0.206
α-tocopherol	3.12	0.014
Lutein	14.28	<0.001
Zeaxanthin	14.02	<0.001
β-cryptoxanthin	2.25	0.600
α-carotene	3.72	0.006
β-carotene	4.73	0.002
13-*cis* β-carotene		

[1]Interaction between group and time
F-value and P-value were calculated by two-way repeated measures ANOVA

Plasma concentrations of both lutein and zeaxanthin significantly changed over time from baseline and these changes were significantly different at each dairy. Plasma lutein concentrations showed significant interactions between group and time (F= 14.28, p <0.001). As illustrated in **Figure 4**, plasma lutein concentrations increased between July and August in cows at the organic dairy (OD), 0.1691ng/dL to 0.3436ng/dL, while plasma lutein concentrations decreased during the same time in cows at the conventional dairy (FD), 0.1013ng/dL to 0.0714ng/dL. Lutein concentrations in cows at both farms decrease from September to November. As expected, plasma lutein concentrations were greater in the plasma of cows from the organic dairy than in the plasma of cows from the conventional dairy over the course of the study.

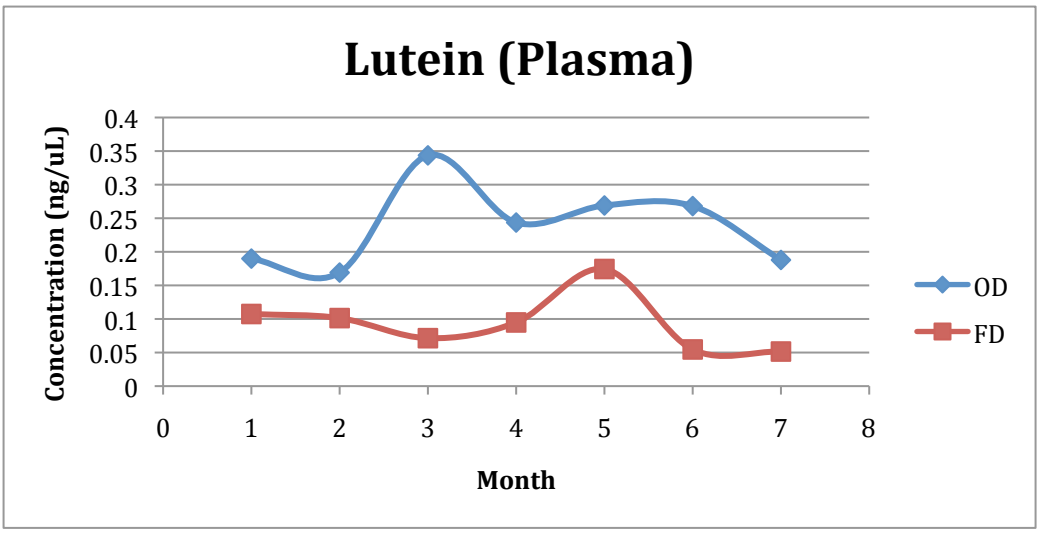


Figure 4. Plasma lutein concentration over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

Plasma zeaxanthin concentrations changed significantly from baseline in different patterns at each dairy ($F = 14.02$, $p < 0.001$). Zeaxanthin concentrations in the plasma decreased in cows at both farms from May to July **(Figure 5)**. As time progressed, plasma zeaxanthin concentrations increased at both farms, however this increase was greater in the plasma of cows at the organic dairy, 0.023931ng/dL to 0.05407ng/dL versus 0.01366ng/dL to 0.02479ng/dL. At the end of the data collection period, the plasma zeaxanthin concentrations in cows at both dairies were similar.

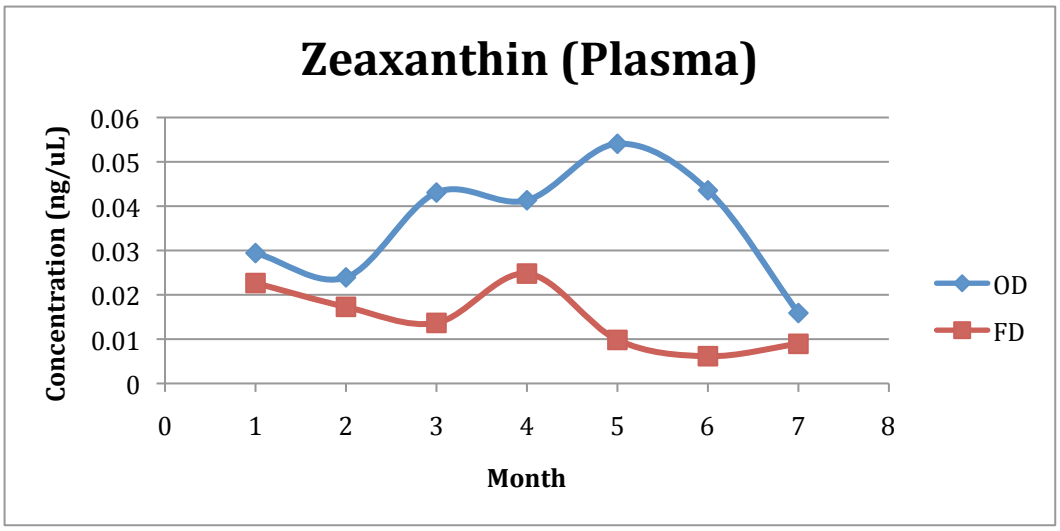


Figure 5. Plasma zeaxanthin concentration over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

Plasma α-carotene and β-carotene concentrations also showed significant interactions between group and time, ($F = 3.72$, $p = 0.006$) and ($F = 4.73$, $p = 0.002$) respectively. α-carotene and β-carotene concentrations in the plasma changed in similar patterns over time and both carotenoid concentrations were greater in cows at the organic dairy than the conventional dairy during the course of the study **(Figure 6 and 7)**.

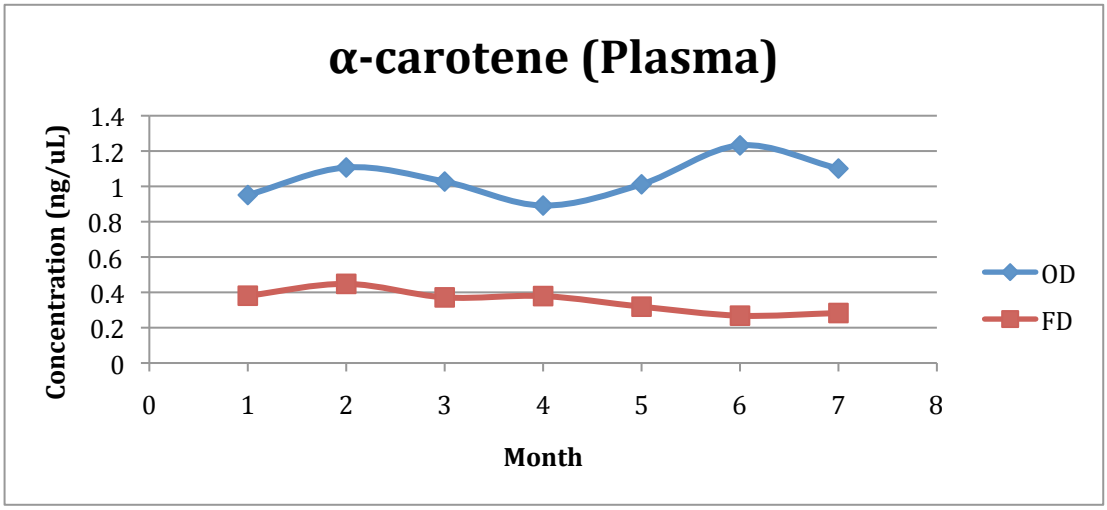


Figure 6. Plasma α-carotene concentration over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

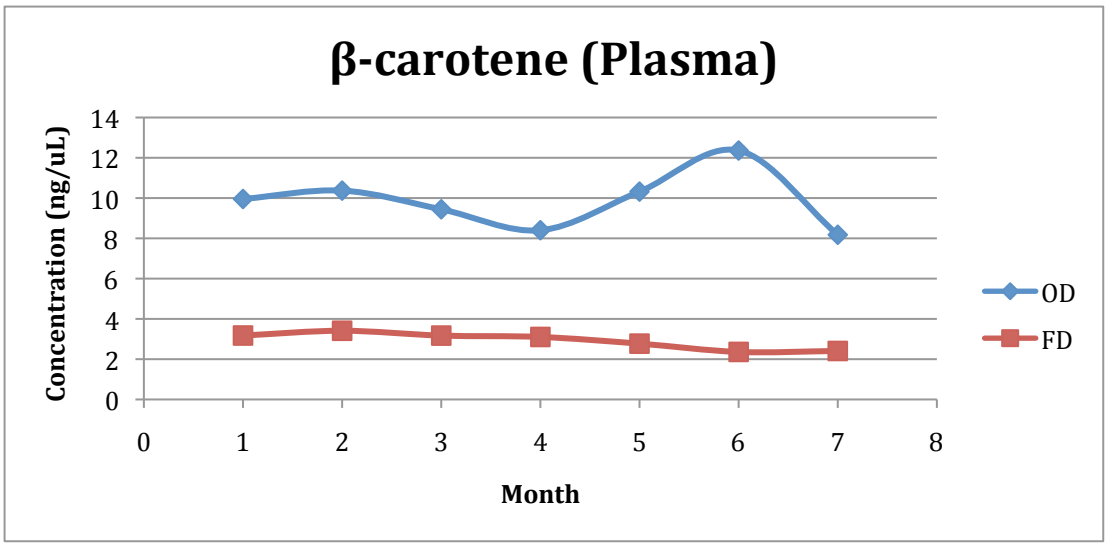


Figure 7. Plasma β-carotene concentration over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

Plasma concentrations of both α-carotene and β-carotene decreased over time at the conventional dairy, 0.381ng/dL to 0.283ng/dL and 3.175ng/dL to 0.283ng/dL respectively. At the organic dairy, plasma α-carotene and β-carotene concentrations decreased in August and peaked in October. Variations in plasma α-tocopherol concentrations were also significantly different at each farm (**Appendix I**).

Discussion

Carotenoids are valuable to human health and are an important part of the human diet. As consumers become more concerned with functional foods, food fortification, and optimizing the nutrient density of foods, it is critical to study the factors that effect the nutrient composition of certain foods. Previous research examined milk and milk products as sources of dietary carotenoids and the potential for varying the amount of carotenoids in these products (21). The current research investigates the changes in the plasma carotenoid concentration of Jersey cows in relation to time, diet, feeding practices, as well as the relationship of the plasma carotenoid concentration to the milk carotenoid concentration.

Many factors have been shown to influence the nutrient content of plasma and milk in cows such as breed, stage of lactation, season, and farming practices. Furthermore, the diet is one of the most significant determinants of the carotenoid concentration in plasma. Evidence shows that a greater consumption of carotenoids through the diet leads to a higher carotenoid concentration in the plasma and ultimately the milk (13, 14, 15). The plasma and milk from cows grazing on pasture has been shown to be composed of different nutrients components than the plasma and milk from cows fed corn and hay forages (13, 14). Thus, the nutrient composition of the plasma and milk changes in coordination with dietary alterations.

Even though the blood is the source of carotenoids for the milk, the conversion of plasma carotenoids to milk carotenoids is not complete, as the blood also provides carotenoids for other biochemical processes in the body. Catranides are also stored in the adipose tissue reducing the amount of these nutrients available for absorption into the milk. Pervious

studies have depicted associations between plasma and milk carotenoids, but the findings are inconclusive. One study showed that 1 to 3% of plasma carotenoids were lutein while only trace amounts were detected in the milk (13). A different study found significant amounts of lutein in the plasma and in the milk reporting that 18% of the total milk carotenoids were attributed to lutein (10). It is important to consider the relationship between plasma and milk carotenoids to better depict the impact of dietary intake on the nutrient composition and carotenoid concentrations of the milk and milk products.

Over the entire study, the 9 Jersey cows from the organic dairy had higher concentrations of plasma lutein, zeaxanthin, α-carotene, and β-carotene than the 9 Jersey cows from the conventional dairy. The cows at the organic dairy grazed on pasture from the end of May to the end of October and supplemented with total mixed ration (TMR). The cows at the conventional dairy were fed exclusively TMR. The composition and nutrient content of the TMR used at each farm were different from one another. It appears that the carotenoid concentration of pasture was higher than the carotenoid concentration of both types of TMR. As expected, the trends were reflected in plasma lutein, zeaxanthin, α-carotene, and β-carotene concentrations over time and season (**Figures 4, 5, 6, and 7**). These results are consistent with findings from previous studies (10, 13).

Plasma concentrations of retinol and β-cryptoxanthin were higher in the plasma from cows at the conventional dairy from May through September (**Figure 8 and 9**). The higher retinol concentration is likely due to the higher concentration of vitamin A in the TMR used at the conventional dairy. As the pasture season progressed at the organic dairy, the retinol or vitamin A concentration in the pasture seemed to surpass the concentration in the

TMR at the conventional dairy. The higher plasma β-cryptoxanthin concentration in cows at the conventional dairy is likely correlated to the higher concentration of corn, a source of this carotenoid, in the TMR at the conventional diary. As time advanced, plasma retinol and β-cryptoxanthin concentrations in cows at the conventional dairy decreased as these plasma concentrations increased in cows at the organic dairy. Due to the variability seen in the plasma retinol and β-cryptoxanthin concentrations, as well as other carotenoid concentrations, in cows at the conventional dairy, it is apparent that the composition of TMR at this dairy changed over the course of the study.

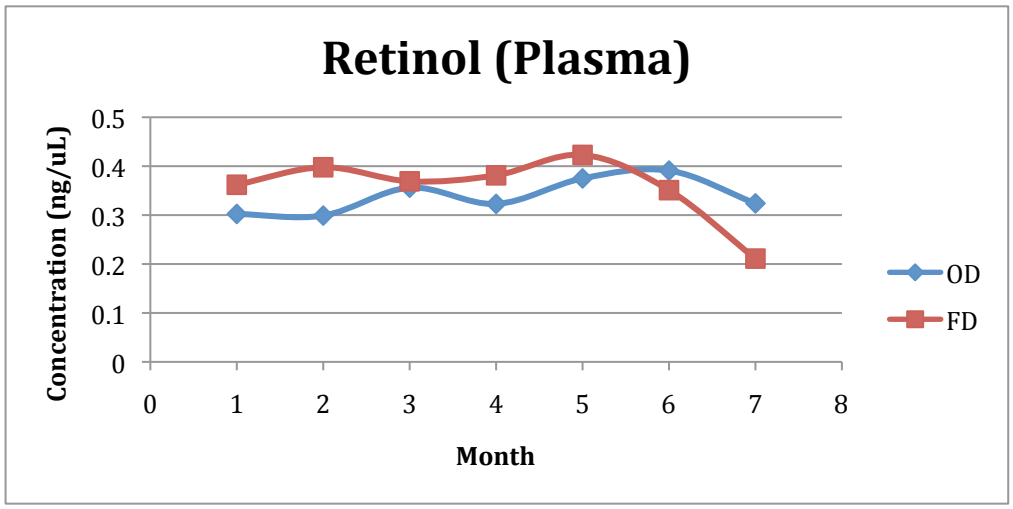


Figure 8. Plasma retinol concentration over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

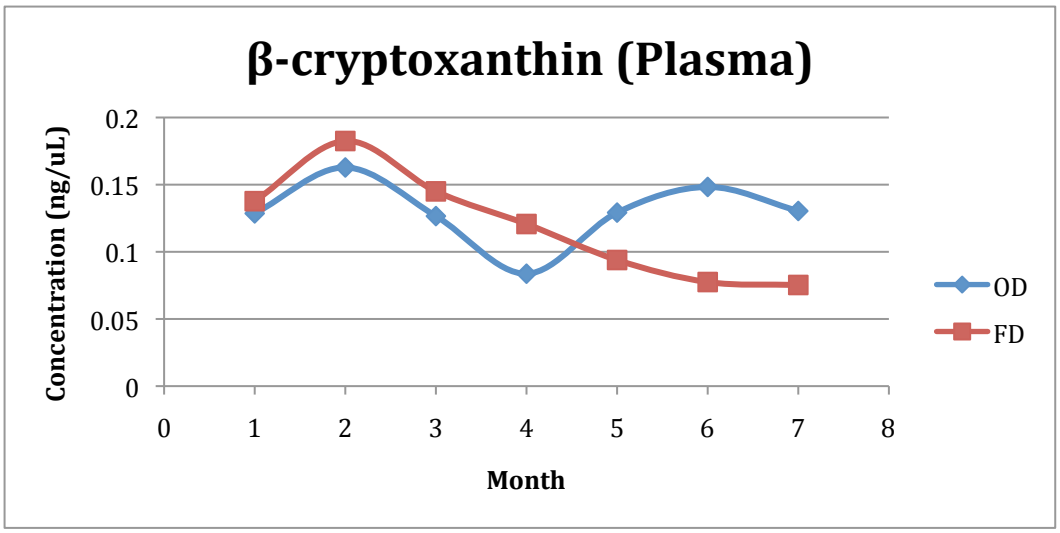


Figure 9. Plasma β-cryptoxanthin concentration over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD

At baseline, plasma β-carotene and 13-*cis* β-carotene concentrations were significantly different in cows at the organic dairy compared to cows at the conventional dairy (**Table 3**). This likely reflects the difference in nutrient compositions of the TMR provided at each farm. There were no significant differences in plasma retinol, α-tocopherol, lutein, zeaxanthin, β-cryptoxanthin, and α-carotene concentrations between cows at the two dairies at baseline. However, after the completion of a pasture season, variations in several plasma carotenoid concentrations (lutein, zeaxanthin, α-carotene, and β-carotene) were significantly different at the organic dairy and the conventional dairy. The consumption of pasture versus TMR had a prolonged effect on the plasma carotenoid concentration of the studied cows.

Plasma lutein, zeaxanthin, α-carotene, and β-carotene concentrations varied over time from baseline. These alterations occurred in significantly different ways at the organic dairy and at the conventional dairy (**Figures 4, 5, 6, and 7**). At the organic dairy, when the cows initiated pasture feeding at the end of May, the plasma lutein, zeaxanthin, α-carotene, and

β-carotene concentrations increased. As the grasses dried out and decreased in nutrient content due to the summer weather, these plasma carotenoid concentrations decreased. When the nutrients in the pasture were restored, an increase in plasma lutein, zeaxanthin, α-carotene, and β-carotene concentrations was observed (**Figure 4, 5, 6, and 7**). As predicted, a decrease in these plasma carotenoid concentrations occurred when the cows were taken off pasture at the end of the pasture season. The seasonal variations of the nutrients in pasture observed in this study are consistent with earlier findings (18).

Lutein, zeaxanthin, α-carotene, and β-carotene concentrations from plasma of cows consuming exclusively TMR did greatly vary over time. Increases in plasma lutein and zeaxanthin concentrations occurred in September at the conventional dairy (**Figure 4 and 5**). This may be indicative of other contributing variables such as the stage of lactation or modifications in the composition of the administered TMR. Plasma α-carotene and β-carotene concentrations remained relatively constant over time (**Figure 6 and 7**).

Furthermore, the plasma carotenoid concentration was compared to the milk carotenoid concentration of the same cows at each dairy facility (21). All of the components of interest in the plasma were detected in the milk. As expected, the concentrations of carotenoids were typically higher in the plasma than in the milk at both dairies. Because the blood supplies carotenoids for many functions in the body, the carotenoid concentration of the plasma is greater than the carotenoid concentration of the milk. The nutrients in the milk are also diluted with fluid, lactose, proteins, and other components at the site of the mammary glands leading to a lower carotenoid concentration (22). Plasma α-tocopherol concentrations and milk α-tocopherol concentrations were comparable, however levels of

α-tocopherol in the plasma were more variable over time. Unlike the carotenoids, retinol concentrations were higher in the milk than in the plasma (**Figure 8 and 10)**. The retinol concentration in the milk is not only dependent on the dietary intake of retinol and the retinol in the blood, but it is also derived from β-carotene and the esterification of the alcohol form of retinol produced by the liver in the mammary glands (16). This process increases the amount of retinol detected in the milk in relation to the plasma.

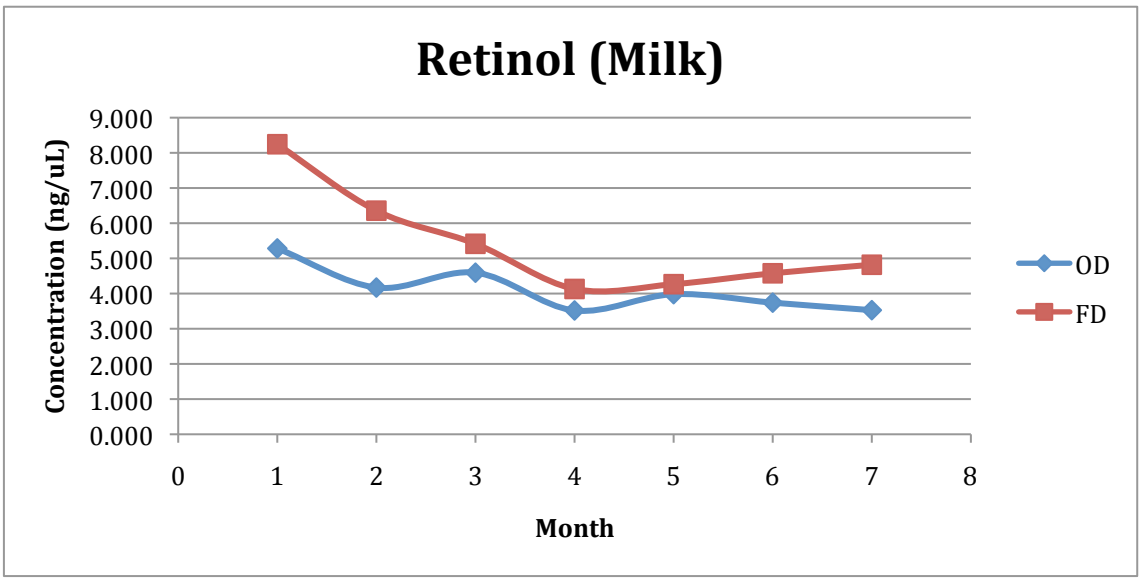


Figure 10. Milk retinol concentration over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

Seasonal variations in milk carotenoid concentrations were also detected, especially in cows from the organic dairy. In cows at the organic dairy, milk lutein and zeaxanthin concentrations decreased initially when pasture feeding began, but then increased through July (**Figure 11 and 12**). In August, there was a depression in milk lutein and zeaxanthin concentrations related to the reduced nutrient content of the pasture grasses. A spike was observed in these carotenoids from September to October when the diet contained a greater quantity of nutrients. Once the cows were removed from pasture and supplemented with

TMR, milk lutein and zeaxanthin concentrations decreased substantially. Milk lutein and zeaxanthin concentrations from cows at the conventional diary did not experience such variations, as their diet of TMR remained relatively stable over time (**Figure 11 and 12**). This similar trend was visualized when comparing plasma lutein and zeaxanthin concentrations at the two dairies.

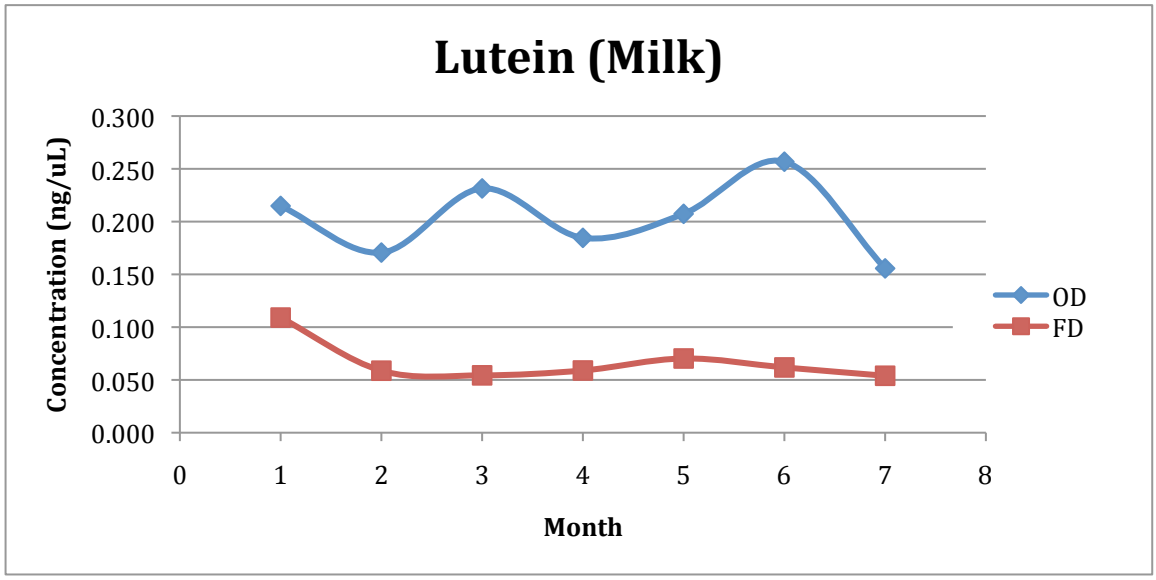


Figure 11. Milk lutein concentration over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

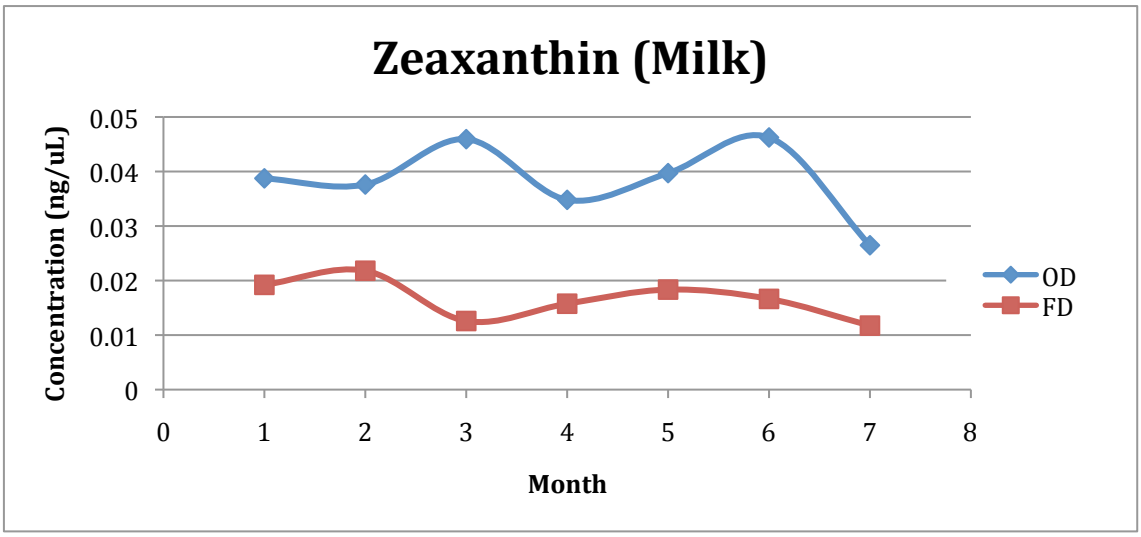


Figure 12. Milk zeaxanthin concentrationn over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

Milk α-carotene and β-carotene concentrations from cows at the organic dairy also varied over time. Unlike milk lutein and zeaxanthin concentrations that took a month to increase once pasture feeding was initiated, as soon as the cows were put onto pasture, milk α-carotene and β-carotene concentrations increased (**Figure 13 and 14**). This may be indicative of the cow's ability to absorb and transport different carotenoids throughout the body. In August, both milk α-carotene and β-carotene concentrations decreased in association with the nutrient content of the pasture grasses. However, these milk carotenoids increased significantly over time in cows that consumed pasture. Concentrations of α-carotene and β-carotene in the milk of cows fed on TMR stayed comparatively constant from May to November (**Figure 13 and 14**).

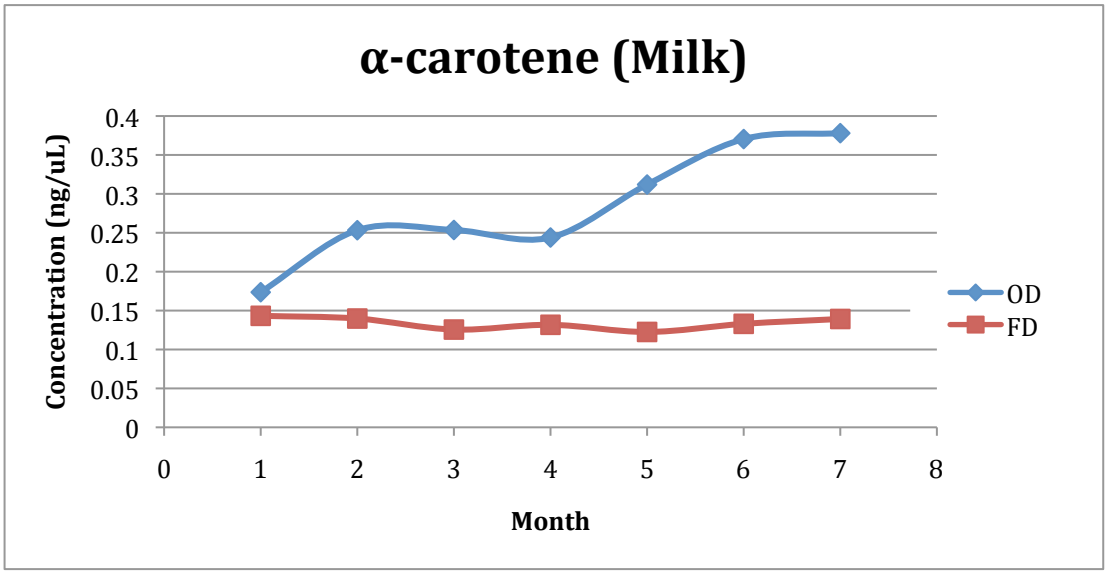


Figure 13. Milk α-carotene concentration over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

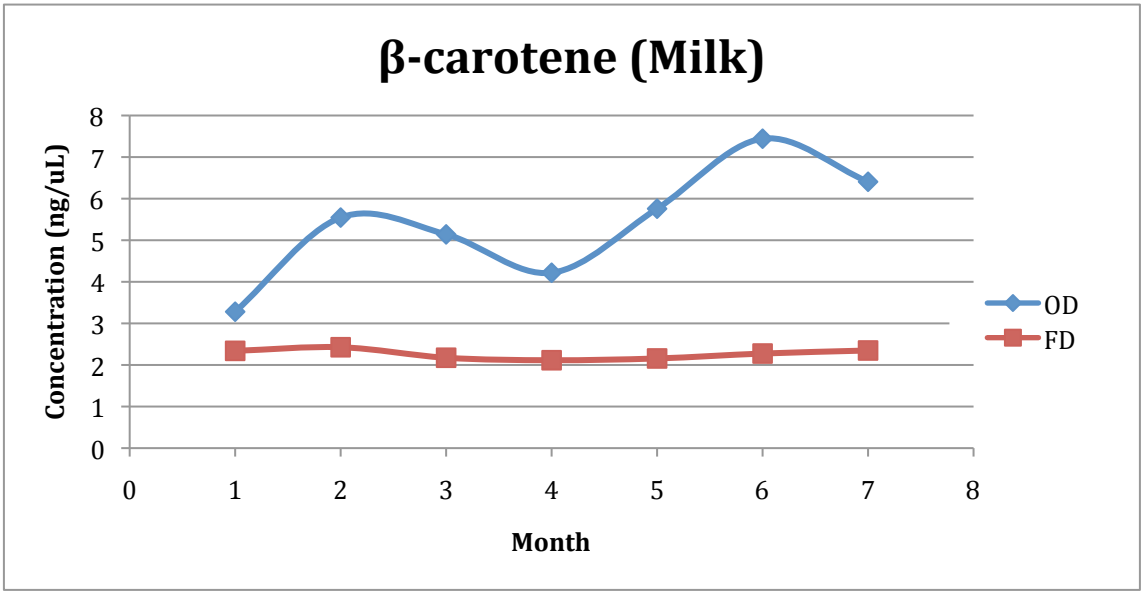


Figure 14. Milk β-carotene concentration over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

It was hypothesized that the trends in plasma and milk carotenoid concentrations over time would be similar, although it was expected that changes in plasma and milk would be syncopated, or in other words, plasma carotenoid concentrations would increase or decrease before milk carotenoid concentrations would. However, this pattern was not illustrated by this study. The results showed that plasma and milk carotenoid concentrations increased or decreased at similar time points. **Figures 15 and 16** show the changes in plasma and milk β-carotene and lutein concentrations respectively over time at both the organic and the conventional dairy. It is evident that the peaks and depressions of the trends for plasma and milk β-carotene and lutein concentrations at the organic dairy occur at similar times. Comparable results were seen for plasma and milk zeaxanthin, β-cryptoxanthin, and α-carotene concentrations at both dairies (**Appendix I**). More studies are needed to investigate the exact trends in data as this data represents changes from month to month and may not be specific enough to gauge week-to-week or day-to-day changes in the plasma and milk carotenoid concentrations.

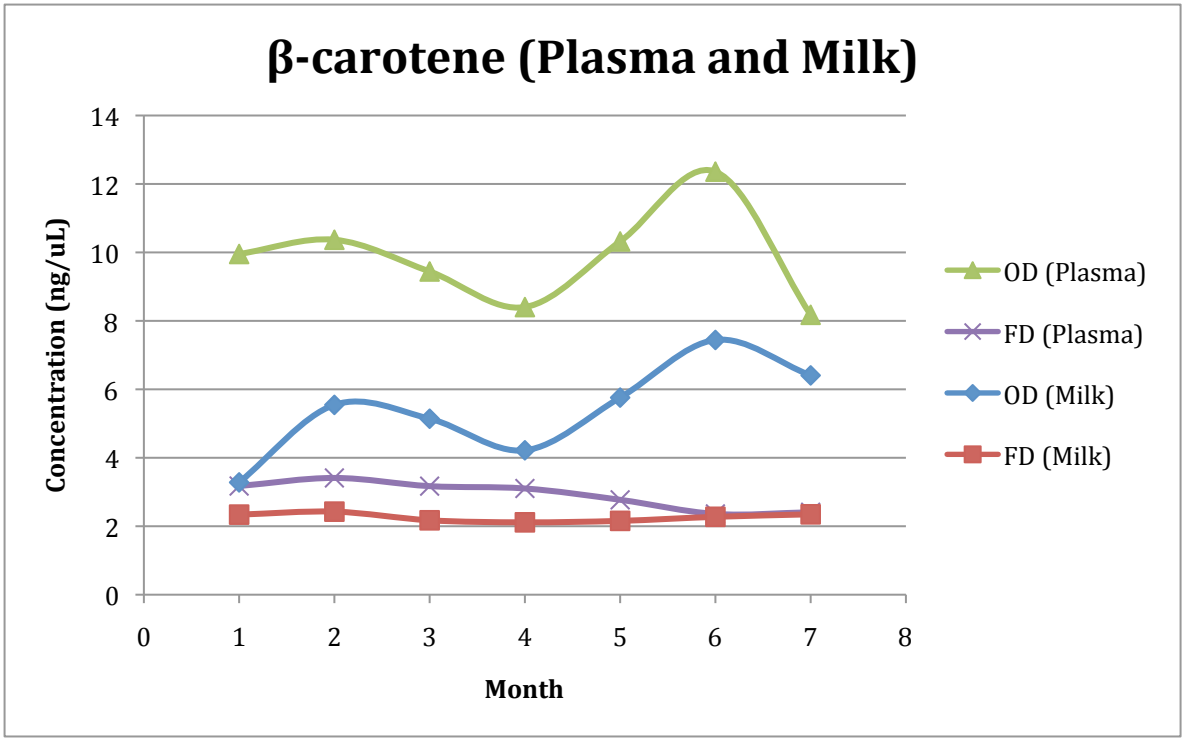


Figure 15. Plasma and milk β-carotene concentrations over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

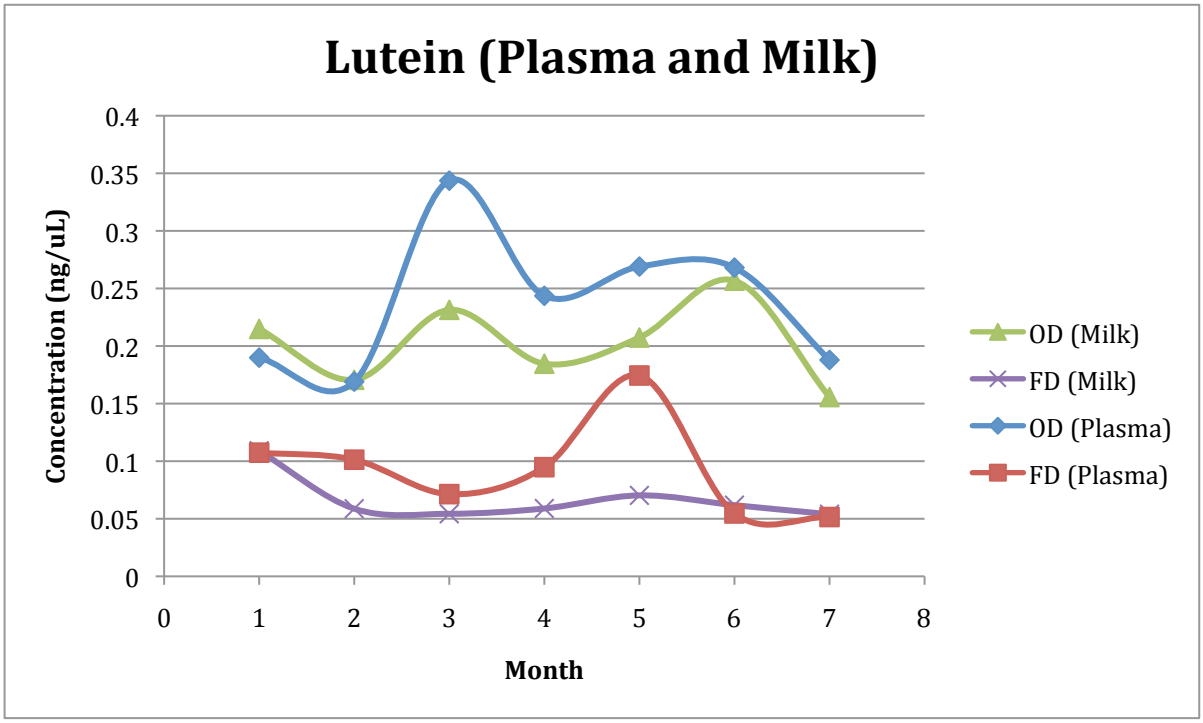


Figure 16. Plasma and milk lutein concentrations over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)

Consumer demand for added value food products is increasing, as people are becoming more interested in the nutrient content and production of food (10, 11). This research is important to better understand the dietary impacts on the nutrient composition of cows' milk. By evaluating nutrient alterations in the plasma over time, inferences can be made about the nutrient content of the milk and milk products. The findings of this study suggest that pasture-based feeding practices increase the carotenoid concentration of the plasma, as well as in milk when compared to feeding practices using TMR. Therefore, increasing the carotenoid concentration of the diet could lead to a higher plasma carotenoid concentration and higher carotenoid concentrations in the milk and the milk products. These products may be beneficial for human health.

This research may also provide useful information for dairy farmers. By increasing the carotenoid concentration of their cows' diets, they could potentially create products that are of higher value to consumers. Because it is shown that consumers want food products that provide added health benefits, they may be more likely to purchase milk with greater nutritional value. The augmentation of milk consumption could profit dairy farmers. Dairy farmers could also subsidize their expenditures through practicing pasture feeding. The increase in pasture intake would decrease the need for other forms of feed like TMR. Further studies are warranted to develop an inclusive understanding of the variability on milk composition related to the consumption of various diets.

Limitations of the Study

Studies have shown that geographical location may influence the content and concentration of nutrients consumed and absorbed by the cows. This data may not be relatable to all international conventional and organic dairy farms. Additionally, various breeds of cows have been shown to process nutrients differently potentially resulting in dissimilar outcomes. The exact composition and nutrient concentration of the pasture grasses and the TMR at each dairy were unknown throughout the study. This lack in data limited the formation of direct correlations between the plasma carotenoid concentration and the carotenoid concentration of the diet. Uncontrolled factors in this study included dairy management practices unrelated to the feed type, stage of lactation, the health of the animals, and weather or seasonal variations. Moreover, the two farms studied in this research are in separate locations greatly increasing the chance for confounding variables.

Conclusion

At baseline, there was no significant difference in plasma retinol, α-tocopherol, lutein, zeaxanthin, β-cryptoxanthin, and α-carotene concentrations between the two dairies. Over time and season, significant changes from baseline ($p<0.05$) in lutein, zeaxanthin, β-cryptoxanthin and β-carotene concentrations, as well as α-tocopherol, occurred at both dairies. At the organic dairy, lutein, zeaxanthin, β-cryptoxanthin and β-carotene concentrations changed significantly by the second month on pasture and continued to change significantly throughout the pasture season. When the cows were taking off pasture in October, the plasma carotenoid concentration decreased significantly. Over the entire study, the 9 Jersey cows from the organic dairy had higher concentrations of plasma lutein, zeaxanthin, α-carotene, and β-carotene than the 9 Jersey cows from the conventional dairy.

All of the components of interest in the plasma were detected in the milk. Seasonal variations in milk lutein, zeaxanthin, α-carotene, and β-carotene were detected over time especially in cows from the organic dairy. As expected, the concentration of carotenoids was typically higher in the plasma than in the milk at both dairies. Changes in milk and plasma carotenoid concentrations were not syncopated. The results showed that plasma and milk carotenoid concentrations increased and decreased at similar time points over the course of the study. More studies are needed to more closely investigate the exact trends in the data.

It is evident that some consumers are interested in the nutritional and sensory quality of their food. An increasing number of people are attracted to food products with added health benefits. Due to the potential health promoting characteristics of carotenoids, increasing the

carotenoid concentrations in milk and milk products would be valuable to the consumer. By utilizing pasture based feeding practices, farmers can economically increase the amount of carotenoids in their products. The results of this study propose that the plasma carotenoid concentration is a good indicator of the milk carotenoid concentration over time. Higher concentrations of carotenoids in the diet lead to an increased plasma carotenoid concentration and therefore greater carotenoid concentrations in the milk and milk products. Further studies are needed to analyze the carotenoid concentrations of the feed and to understand the mechanisms by which the carotenoids are extracted from the food supply, absorbed by the body into the blood stream, and excreted in the milk.

References

1. Britton G. Structure and properties of carotenoids in relation to function. FASEB J. 1995;9:1551.

2. Rao A, Rao L. Carotenoids and human health. Pharmacol Res. 2007;55:207.

3. Mares-Perlman J, Millen A, Ficek T, Hankinson S. The Body of Evidence to Support a Protective Role for Lutein and Zeaxanthin in Delaying Chronic Disease. Overview. J Nutr. 2002;132:518S.

4. Mimuro ,M., Katoh T. Carotenoids in photosynthesis: absorption, transfer and dissipation of light energy. Pure Appl Chem. 1991;63:123.

5. Thane C, Reedy S. Processing of fruit and vegetables: effect on carotenoid. Nutr Food Sci. 1997;92:58.

6. Halliwell B. Free Radicals and Antioxidants: A Personal View. Nutr Rev. 1994;52:253.

7. Tang G. Bioconversion of dietary provitamin A carotenoids to vitamin A in humans. Am J Clin Nutr. 2010;91:1468S.

8. Miki W. Biological functions and activities of animal carotenoids. Pure Appl Chem. 1991;63:141.

9. Veeramachaneni S, Wang X. Carotenoids and lung cancer prevention. Front Biosci. 2009;S1:258.

10. Calderon F, Chauveau-Duriot B, Martin B, Graulet B, Doreau M, Noziere P. Variations in Carotenoids, Vitamin A and E, and Color in Cow's Plasma and Milk During Late Pregnancy and the First Three Months of Lactation. J Dairy Sci. 2007;90:2335.

11. Douthitt R. Consumer Risk Perception and Recombinant Bovine Growth Hormone: The Case for Labeling Dairy Products Made from Untreated Herd Milk. J Public Policy Mark. 1995;14:328.

12. Sebastian R, Goldmand D, Enns C, Lacomb R. Fluid milk consumption in the united states. Data Brief. USDA; 2010. Report No.: 3.

13. Noziere, P., Grolier, P., Durand, T., Ferlay, A., Pradel, P., Martin, A. Variations in Carotenoids, Fat-Soluble Micronutrients, and Color in Cows' Plasma and Milk Following Changes in Forage and Feeding Level. J Dairy Sci. 2006;89:2634.

14. Slots T, Butler G, Leifert C, Kristensen T, Skibsted L, Nielsen J. Potentials to differentiate milk composition by different feeding strategies. J Dairy Sci. 2009;92:2057.

15. Baumann C, Steenbock H, Beeson W, Rupel I. The influence of breed and diet of cows on the carotene and vitamin A content of butter. J Boil Chem. 1934;105:167.

16. Noziere P, Graulet B, Lucas A, Martin B, Grolier P, Doreau M. Carotenoids for ruminants: From forages to dairy products. Anim Feed Sci Technolo. 2006;131:418.

17. Hulshof, P., Roekel-Jansen, T., Bovenkamp, P., West, C. Variations in retinol and carotenoid content of milk and milk products in The Netherlands. J Food Compos Anal. 2006;19:67.

18. Chihuailaf R, Gonzalez C, Wittwer F, Contreras P. Plasma retinol concentration in grazing heifers: First data obtained from a dairy herd in the south of Chile. Arch Med Vet. 2008;40:65.

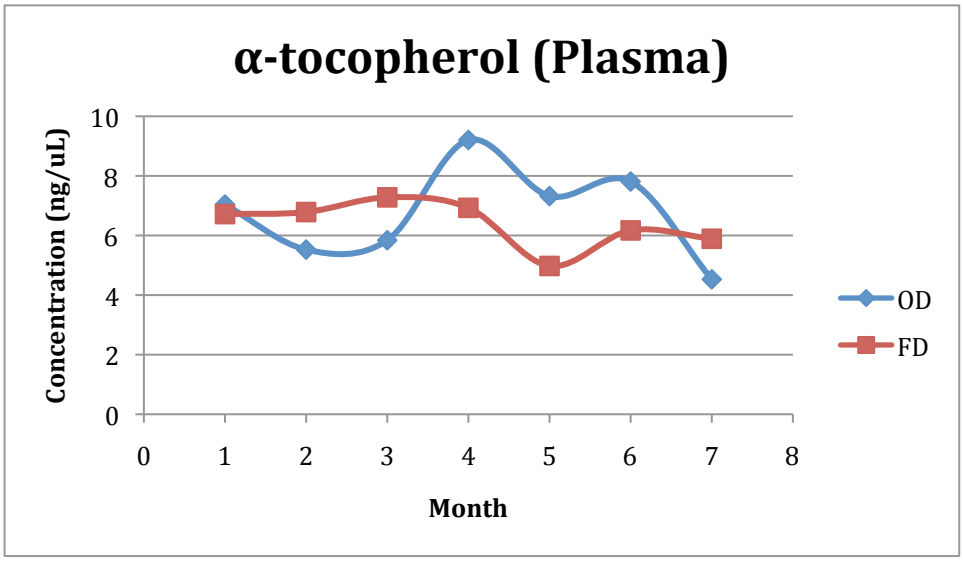
19. Handelman G, Shen B, Krinksy N. High resolution analysis of carotenoids in human plasma by high-performance liquid chromatography. Method Enzymol. 1991;213:336.

20. Jin-Yeum K, et al. Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetable. Am J Clin Nutr. 1996;64:594.

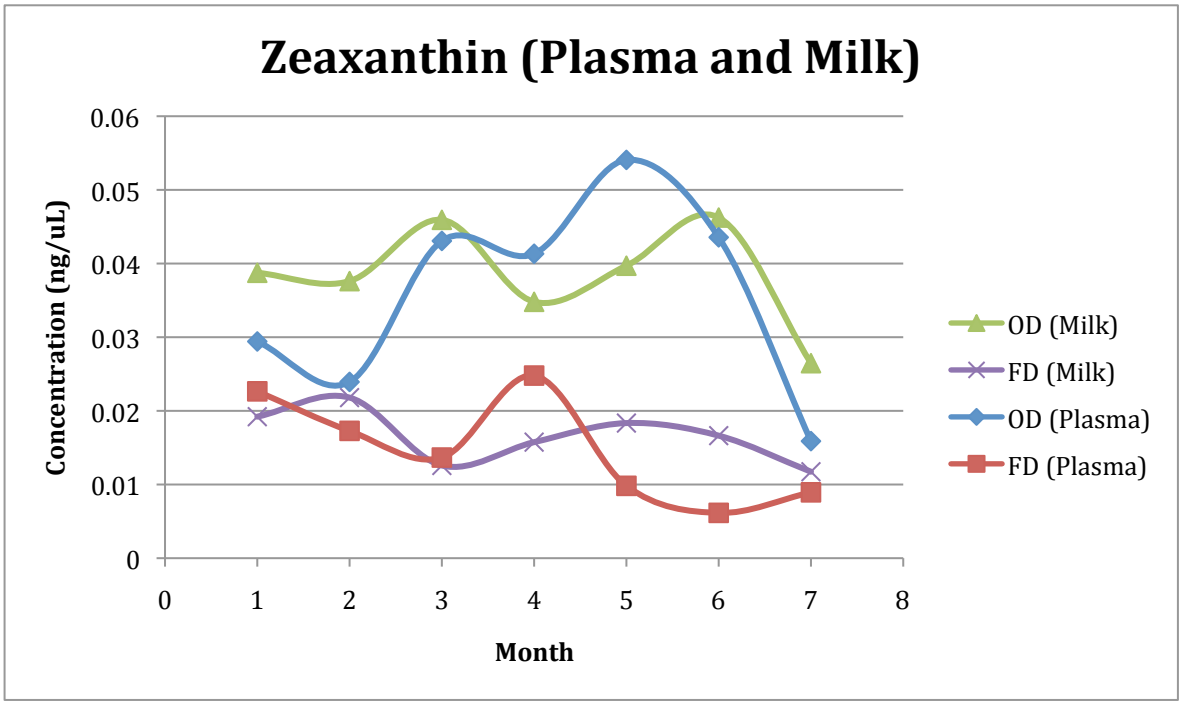
21. Beliveau A. Variations in carotenoids and retinol in milk and cheese from jersey cows at an organic dairy compared to a conventional dairy over a pasture season [dissertation]. University of New Hampshire; 2012.

22. Akers RM. Lactation and the mammary gland. 1st ed. Iowa: Iowa State Press, 2002.

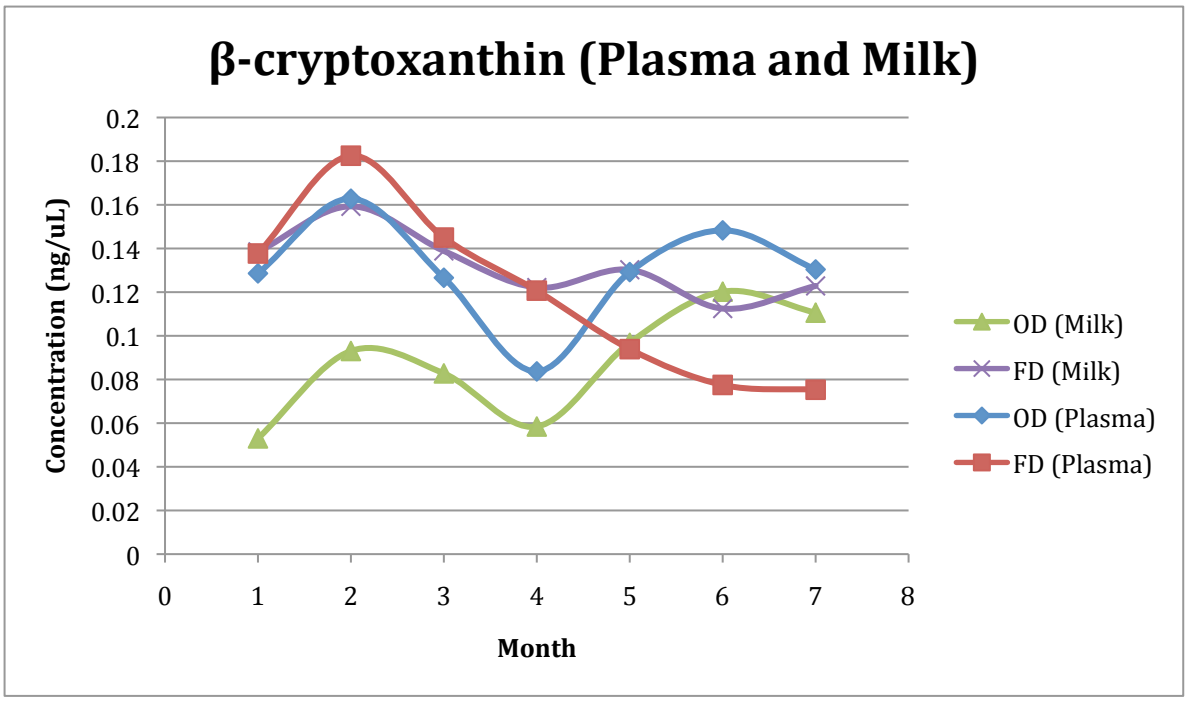
Appendix I



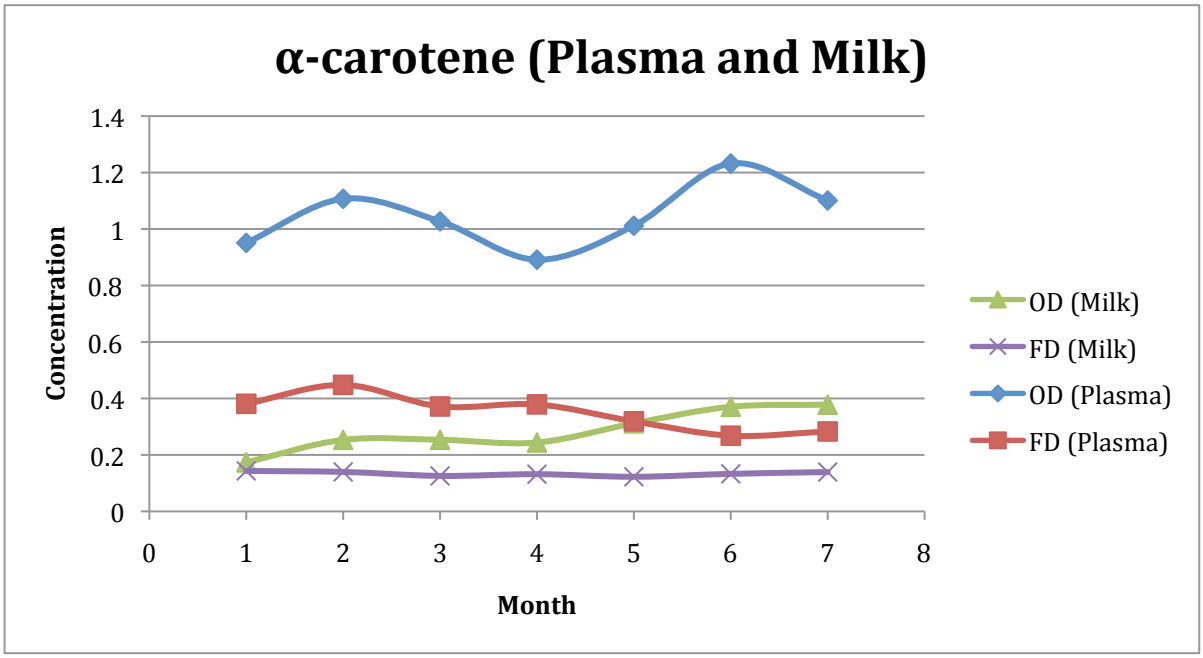
Plasma α-tocopherol concentration over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)



Plasma and milk zeaxanthin concentrations over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)



Plasma and milk β-cryptoxanthin concentrations over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)



Plasma and milk α-carotene concentrations over time at the UNH Fairchild Dairy Teaching and Research Center (FD) and the UNH Organic Dairy Research Facility (OD)