Soil Nutrient Management Practices Influence the Carotenoid Content and Profile of Orange Fleshed Sweet Potato Variety (UMUSPO 3)

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Abstract. Food bio fortification holds a lot of promise for the alleviation of hidden hunger. The appropriate soil nutrient management methods that will maximize the pro vitamin A content of bio fortified Orange fleshed sweet potatoes (OFSP) (Umudike Sweet Potato Orange-fleshed 3) was evaluated in the present research. The carotenoid content and profile of OFSP variety grown on soils given five different nutrient managements were compared. The soil management treatments given were: (VB₁) Poultry manure at 5 x 10³ kg/ha (control); VB₂: Poultry manure at 2.5 x 10³ kg/ha + NPK at 200 kg/ha + Agrolyser at 2.7 kg/ha, VB₃: Agrolyser at 2.7 kg/ha + NPK at 200 kg/ha; VB₄: Poultry manure at 5.0 x 10³ kg/ha + NPK at 200 kg/ha, VB₅: NPK 15:15:15 at 400 kg/ha. (Agrolyser: Ca – 20.4%; Na – 1.04%; Zn – 0.11%, S – 2.72%; Fe, Mn, Mo trace) (NPK 15:15:15 was used in all cases).

Carotenoids were extracted from the potato samples and analyzed using High performance Liquid Chromatography (HPLC). The best treatment that promoted high pro vitamin A carotenoid content was soil treatment with Poultry manure (5x 10³ kg/ha+ NPK (200kg/ha). This soil treatment led to the highest contents of α -carotene (6.14 μ g/g); 13-cis- β -carotene (12.36 μ g/g); All-trans- β -carotene (87.89 μ g/g) and 9-cis- β -carotene (2.99 μ g/g). The best soil management treatment for the highest yield of β -cryptoxanthin (7.95 μ g/g) was poultry manure at 2.5kg/ha +NPK at 200kg/ha.

Introduction

Vitamin A deficiency (VAD) is considered a serious problem of public health significance in over 70 countries. Countries of South-East Asia have the highest prevalence of VAD (49.9%) followed by Africa, where 44.4% of preschool-age children and 13.5% of pregnant women suffer from vitamin A deficiency [1]. It is an essential micronutrient for normal immune function of the body. It is essential for good health and eyesight [2]. The dietary sources of vitamin A are preformed vitamin A which comes from animal origin (such as fish oils, liver, milk, eggs and butter) that contain vitamin A in its true form (also called retinol) which can be used directly and easily by the human body and also pro-vitamin A carotenoids (found in yellow and orange-fleshed fruit and vegetables and in dark-green leafy vegetables) [3, 4]. Of the approximately 600 carotenoids found in nature, only three are important precursors of vitamin A in humans beings viz, β -carotene, α -carotene and β -cryptoxanthin [5] and β -carotene is the major pro-vitamin A of most carotenoid containing foods [6]. Recent studies associated with the consumption of carotenoid rich food showed the decrease of the incidence of certain cancers in human beings [7]. Food fortification, food bio-fortification, dietary diversification and vitamin A supplementation are the recommended strategies to control vitamin A deficiency [8]. Orange-Fleshed Sweet Potato (OFSP) forms an important source of carotene rich food [9, 10]. Jailal *et al.* [11] suggested that a food based approach may be a successful way of reducing the prevalence of vitamin A deficiency [12]. One medium sized OFSP (depending on the carotenoid content of the OFSP) is said to provide about twice the β -carotene needed for the recommended daily requirement of vitamin A. Vitamin A deficiency prevents people from surviving and thriving as

productive members of society and stagnates countries in a cycle of poor nutrition, poor health, lost productivity, persistent poverty, and reduced economic growth [13].

Sweet potato, (*Ipomea batatas* Lam.), is an important staple food crop in Nigeria [14]. It grows fast and requires low work input. Sweet potato is often planted in Africa as a security crop or famine prevention crop [10]. The main sweet potato producers are China, Indonesia, Vietnam, India, Philippines and Japan in Asia, Brazil and the USA the Americas and Nigeria, Uganda, etc [15]. It has the potentials to improve household and national food security, health and livelihoods of poor families in sub-Saharan Africa [16]. It is an important alternative source of carbohydrates consumed in the country as an energy giving food and attains the fourth position after rice, corn and cassava. Presently, this crop is considered as having low economic value but it has significant social importance. It is very popularly used as snack food, but it is also used as staple food or as a rice substitute in many countries [17]. It is high in carbohydrate and vitamin A contents (some varieties) and can produce more edible energy per hectare per day than wheat, rice, or cassava. The roots may be processed into starch, candy, flour and alcohol. The leaves are edible (Belehu, 2003) [18]. Figure 1 shows UMUSPO3 Orange fleshed sweet potato variety.

Figure1. UMUSPO 3 Orange-fleshed sweet potato variety

Field Work, Experimental Design and Treatments

The field work was carried out by the agronomy students of Michael Okpara University of Agriculture, Umudike. The sweet potato variety (UMUSPO 3) was planted at the National Root Crops Research Institute, experimental farm, Umudike, Abia State, Nigeria (situated between latitude 05°29'N and longitude 07°33' E and 122m altitude). The soil is sandy loam, 78.8% sand, 6.8% silt, 14.4% clay, pH 4.7, 1.59% organic matter, 0.08% Nitrogen (N); 32.1 mg/kg Phosphorus (P), and 0.35 mg/kg, Potassium (K) (as determined using standard AOAC methods). The treatments were arranged in randomized complete block design with three replications. The main plot treatment was one variety (UMUSPO 3) of Orange-Fleshed Sweet Potatoes (OFSP). The treatments were five nutrient combinations with control. (VB₁) Poultry manure at 5 x 10³ kg/ha (control); VB₂: Poultry manure at 2.5 x 10³ kg/ha + NPK at 200 kg/ha + Agrolyser at 2.7 kg/ha, VB₃: Agrolyser at 2.7 kg/ha + NPK at 200 kg/ha; VB₄: Poultry manure at 5.0 x 10³ kg/ha + NPK at 200 kg/ha, VB₅: NPK 15:15:15 at 400 kg/ha. (Agrolyser: Ca – 20.4%; Na – 1.04%; Zn – 0.11%, S – 2.72%; Fe, Mn, Mo trace) (NPK 15:15:15) was used in all cases.

Chemical analysis showed that the poultry manure (measured using standard AOAC methods) had values of pH 7.06, N 2.17%, P 1.06% and K 0.62% as the total content of samples. The poultry manure were applied into appropriate plots after ridging while NPK were applied 4 weeks after planting by band placement. Each sub-plot measured 3 m x 2 m (6 m²). Sweet potato vine cuttings of 20 cm length with at least 4 nodes were planted during the rainy season (Mid July) at a distance of 30 cm apart, along the crest of the ridges. Supply of vacant stands was done at 2 weeks after planting.

Determination of Carotenoids

The analysis of the β -carotene in sweet potato samples was carried out at the Crop Utilization Unit (Laboratory) of International Institute of Tropical Agriculture (IITA) Ibadan. The method of Rodriguez-Amaya and Kimura [19] was used.

Sample preparation: Fresh sweet potato tubers (UMUSPO 3) were selected randomly and washed with clean water. They were peeled, washed and sectioned into four longitudinally. Two opposite

sections from each tuber were taken, sliced into small pieces (1 cm) and mixed manually. They were packaged in aluminum foil, labeled and stored at -80°C in a deep freezer.

Extraction: Carotenoids were extracted by grinding about 3 g of each sample in a mortar and pestle with about 50 ml of cold acetone. The residue was filtered in a Buchner funnel layered with a filter paper (Whatman No. 42 filter paper). The residue was returned to the mortar and the extraction was repeated using 20 ml acetone until the residue was nearly colourless. The total extract was transferred into a separating funnel (250 ml) containing 20 ml of petroleum ether. One litre of distilled water was used to wash the organic phase which separated from the aqueous phase. The aqueous phase was discarded. The organic phase was again washed with dilute brine solution to break-up any formed emulsions. The organic phase was collected through anhydrous Sodium sulphate (15 g) into a 25 ml flat bottom flask. Ten (10) ml of the sample extract was concentrated with a rotary evaporator (Buchi Waterbath B-481 Switzerland) and dried under vacuum, for reverse-phase HPLC determinations of the various carotenoid fractions.

HPLC analysis: The modified method of Howe and Tanumihardjo [20] was used for the HPLC analysis. A quantity of 15 ml of the freeze dried samples was reconstituted in methanol/dichloroethane (1 ml, 50:50 v/v) and 50 µl was injected into the HPLC. A water HPLC system (Water Corporation, Milford, MA) consisting of a guard column, C30YMC carotenoid column (4.6 x 250 mm, 3 µm) water 626 binary HPLC pump, 717 auto sampler and a 2996 photodiode array detector was used for carotenoids quantification. Solvent A was 100% methanol, while solvent B was 100% methyl tert-butyl ether (MTBE). Isocratic elution was carried out at 1 ml/min while the run time was 15 min.

Spectrophotometric reading and calculation: Absorbance of carotenoid ether extract was taken at 450 nm using a spectrophotometer. The formula described by Rodriguez-Amaya and Kimura [19] was used to quantify the carotenoid content:

$$C_x (\mu\text{g/g}) = \frac{A_x \times C_s (\mu\text{g/ml}) \times \text{total Vol. of extract (ml)}}{A_s \times \text{sample weight (g)}}$$

Where:

C_x = Concentration (µg/g) of carotenoid

A_x = Peak Area of the carotenoid

C_s = Concentration of standard

A_s = Peak area of the standard

The standard curve already established for the HPLC was used. The remaining 15 ml sample extract was used to determine the total carotenoids spectrophotometrically.

The absorbance was measured at 450 nm in a spectrophotometer. The total carotenoids content was calculated with the formula

$$\text{Total carotenoids content } (\mu\text{g/g}) = \frac{A \times \text{Vol. (ml)} \times 10^4}{A^{1\%} \times \text{sample weight (g)}}$$

Where:

A = Absorbance

Vol. = Total volume of extract (25 ml)

$A^{1\%}$ = Absorption coefficient of β -carotene in PE (2592)

The result was multiplied by 100 to obtain the carotenoid content in µg/100 g. All sample preparations, extractions and analysis were performed under white fluorescent lighting.

Data Analysis

Analysis of variance and multiple range test were used for the determination of significant differences ($p < 0.05$) among treatment means and separation of means was carried out using the SPSS

17 software. Separation of means was carried out by Duncan Multiple range test and values were reported as means and standard deviation.

Pro-Vitamin A Content of UMUSPO 3 *Ipomea Batatas* Variety (FW)

Table 2 shows the Pro-vitamin A carotenoids and retinol equivalents of UMUSPO 3 *Ipomea batatas* variety.

Pro-vitamin A carotenoids: β -cryptoxanthin had the mean values of 6.41 $\mu\text{g/g}$ was highest (7.95 $\mu\text{g/g}$) in the sample grown on soil treated with poultry manure at 2.5×10^3 kg/ha plus NPK at 200 kg/ha plus Agrolyser at 2.7 kg/ha (VB₂). This was significantly different ($p < 0.05$) from other samples. The lowest value (3.09 $\mu\text{g/g}$) was obtained from *Ipomea batatas* planted on poultry manure treated soil at 5.0×10^3 kg/ha (VB₁). β -cryptoxanthin exhibit 50% pro-vitamin A activity of β -carotene [21,22]. α -carotene contents of the samples varied significantly ($p < 0.05$) with treatments. The highest value (6.14 $\mu\text{g/g}$) was from the sample grown on soil treated with poultry manure at 5.0×10^3 kg/ha plus NPK at 400 kg/ha (VB₄), while the *Ipomea batatas* grown on soil treated with Agrolyser at 2.7 t/ha plus NPK at 200 kg/ha (VB₃) had the lowest value (2.5 $\mu\text{g/g}$). α -carotene has an unsubstituted β -ring and nine conjugated double bonds thus it exhibits half of the pro-vitamin A activity of β -carotene [19,23].

13-Cis- and 9-Cis- β -carotene values varied significantly ($p < 0.05$) with different soil nutrient managements. The highest carotenoid contents were 12.36 $\mu\text{g/g}$ and 2.99 $\mu\text{g/g}$ for 13-Cis- β -carotene and 9-Cis- β -carotene respectively obtained from *Ipomea batatas* planted on soil treated with poultry manure at 5.0×10^3 kg/ha plus NPK at 200 kg/ha (VB₄).

Nitrogen fertilizer application above 80 kg N/ha has been reported as not beneficial for beta -carotene yield for many sweet potato varieties ($P > 0.05$) except for CIP Tanzania [22]. This research indicates that for UMUSPO3 OFSP, nitrogen levels above 80kg N/ha are beneficial. Beta-carotene rich sweet potatoes have the potential to improve vitamin A status among the vulnerable groups in developing countries [22]. Marked variations have been observed in chemical constituents of sweet potato varieties [24]. These variations are invariably influenced by soil nutrients. Kalu et al. [25] investigated the effect of planting distance and harvest period on the carotenoid retention of this same sweet potato variety (UMUSPO3). A planting distance of 40 cm apart supported the maximum yield of 13-cis-beta carotene. This OFSP variety has also been found to be rich in carbohydrates and reducing sugars [26].

Table 2. Effect of different nutrient management treatments on pro-vitamin A content of UMUSPO 3 *Ipomea batatas* variety (FW)

	VB ₁	VB ₂	VB ₃	VB ₄	VB ₅
β -cryptoxanthin ($\mu\text{g/g}$)	3.091 ^e ±0.00	7.95 ^a ±0.01	5.93 ^d ±0.01	7.32 ^c ±0.06	7.76 ^b ±0.02
α -carotene ($\mu\text{g/g}$)	3.41 ^b ±0.04	3.17 ^d ±0.01	2.56 ^e ±0.01	6.14 ^a ±0.10	3.28 ^c ±0.01
13-Cis- β -carotene ($\mu\text{g/g}$)	10.81 ^b ±0.13	3.56 ^d ±0.04	3.17 ^e ±0.04	12.36 ^a ±0.21	3.92 ^c ±0.04
All-Trans- β -carotene ($\mu\text{g/g}$)	64.25 ^e ±0.03	84.41 ^c ±0.06	76.78 ^d ±0.10	87.89 ^a ±0.18	85.67 ^b ±0.12
9-Cis- β -carotene ($\mu\text{g/g}$)	1.73 ^d ±0.02	1.93 ^b ±0.05	1.31 ^e ±0.01	2.99 ^a ±0.01	1.82 ^c ±0.01
Vitamin A (RE/100 g Fw)	1230	1545	1388	1705	1568

Values are mean± SD. Means with different superscript in the same column are significantly different ($p < 0.05$) VB₁: Poultry manure at 5×10^3 kg/ha; VB₂: Poultry manure at 2.5×10^3 kg/ha + NPK at 200 kg/ha + Agrolyser at 2.7 kg/ha, VB₃: Agrolyser at 2.7 kg/ha + NPK at 200 kg/ha; VB₄: Poultry manure at 5.0×10^3 kg/ha + NPK at 200 kg/ha, VB₅: NPK 15:15:15 at 400 kg/ha

The lowest values for the two Cis-isomers, 3.17 µg/g and 1.31 µg/g for 13-Cis-β-carotene and 9-Cis-β-carotene were obtained from samples grown on soil treated with Agrolyzer at 2.7 kg/ha NPK plus at 200 kg/ha (VB₃).

Table 3. Chromatogram data for *Ipomea batatas* (Variety UMUSPO 3) treated with poultry manure at 5 t/ha (VB₁)

Peak Number	Retention time (min)	%Area	Peak Number	Retention time (min)	%Area
1	2,098	0,05	10	4,240	0,62
2	2,847	0,05	11	4,502	3,44
3	3,028	0,14	12	4,923	3,53
4	3,140	0,31	13	5,228	12,39
5	3,424	0,94	14	5,896	75,62
6	3,567	0,10	15	6,620	1,89
7	3,751	1,13	16	7,227	0,08
8	3,937	1,53	17	8,052	0,08
9	4,133	0,09			

Table 3 shows the data for the UMUSPO 3 *Ipomea batatas* variety grown on soil subjected to poultry manure treatment at 5.0 x 10³ kg/ha (VB₁). Seven out of seventeen eluted carotenoid fractions were identified and quantified. Trans-β-carotene predominated with 64.25 µg/g (73.62%) carotenoid content, followed by 13-Cis-β-carotene (0.81 µg/g) (12.39%).

α-carotene had the concentration of (3.41 µg/g) (3.53%), β-cryptoxanthin had 3.09 µg/g (3.44%), 9-Cis-β-carotene had 1.73 µg/g (1.89%), zeaxanthin had 1.53 µg/g (1.53%), while lutein recorded 1.15 µg/g (1.13%). The higher carotenoid values of this variety (UMUSPO 3) correspond with their peak areas which seem to be higher than UMUSPO 1 *Ipomea batatas* variety.

Table 4. Chromatogram data for *Ipomea batatas* (Variety UMUSPO 3) treated with poultry manure at 2.5 t/ha plus NPK at 200 kg/ha plus Agrolyser at 2.7 kg/ha (VB₂)

Peak Number	Retention time (min)	%Area	Peak Number	Retention time (min)	%Area
1	2,130	0,17	8	4,493	7,31
2	2,806	0,07	9	4,912	2,63
3	3,117	0,48	10	5,218	3,18
4	3,420	1,25	11	5,886	77,98
5	3,729	2,23	12	6,609	1,72
6	3,928	2,23	13	7,252	0,06
7	4,235	0,55	14	8,022	0,05

Table 4 shows the data for the UMUSPO 3 *Ipomea batatas* variety treated with poultry manure at 2.5 x 10³ kg/ha plus NPK at 200 kg/ha plus Agrolyser 2.7 kg/ha. The total of fourteen carotenoid fractions eluted while seven were identified and quantified. The predominant trans-β-carotene had the concentration of 84.41 µg/g (77.98%). β-cryptoxanthin and 13-Cis-β-carotene followed with 7.95 µg/g (7.31%) and 3.56 µg/g (3.18%) concentrations respectively, while α-carotene had 3.17 µg/g, (2.63%) , zeaxanthin had 3.00 µg/g (2.33%), Lutein yielded 2.85 µg/g (2.23%) and 9-Cis-β-carotene had 1.93 µg/g (1.72%) carotenoid concentrations.

Table 5. Chromatogram data for *Ipomea batatas* (Variety: UMUSPO 3) treated with Agrolyser at 2.7 kg/ha (VB3)

Peak Number	Retention time (min)	%Area	Peak Number	Retention time (min)	%Area
1	2,098	0,07	8	4,495	6,17
2	2,812	0,06	9	4,915	2,41
3	3,122	0,47	10	5,220	3,20
4	3,423	1,11	11	5,892	80,76
5	3,735	1,95	12	6,605	1,27
6	3,932	2,00	13	7,256	0,06
7	4,240	0,43	14	8,036	0,04

Table 5 shows data for the UMUSPO 3 *Ipomea batatas* variety treated with Agrolyser at 2.7 kg/ha plus NPK at 200 kg/ha. A total of fourteen carotenoids eluted and separated but only seven major carotenoids were identified and quantified. The predominant trans- β -carotene had the concentration of 76.78 $\mu\text{g/g}$, (80.76%), followed by β -cryptoxanthin with 5.93 $\mu\text{g/g}$ (6.17%), 13-Cis- β -carotene 3.17 $\mu\text{g/g}$ (3.20%) concentration whereas α -carotene, zeaxanthin, lutein and 9-Cis- β -carotene had 2.56 $\mu\text{g/g}$ (2.41%), 2.25 $\mu\text{g/g}$ (2.0%), 2.18 $\mu\text{g/g}$, (1.95%), and 1.31 $\mu\text{g/g}$ (1.27%) carotenoid contents. It is worthy to note that trans- β -carotene and its Cis-isomers accounted for about 85.23% of the total of the carotenoids leaving only 14.77% to other minor carotenoids.

Table 6. Chromatogram data for *Ipomea batatas* (Variety: UMUSPO 3) treated with poultry manure at 5 t/ha plus NPK at 200 kg/ha (VB4)

Peak Number	Retention time (min)	%Area	Peak Number	Retention time (min)	%Area
1	2,81	0,04	8	4,49	5,84
2	3,15	0,48	9	4,922	4,42
3	3,42	1,30	10	5,224	9,80
4	3,736	1,30	11	5,883	70,96
5	3,934	1,38	12	6,617	2,34
6	4,078	0,93	13	7,224	0,10
7	4,235	0,70	14	8,038	0,09

Table 6 is the data for the UMUSPO 3 *Ipomea batatas* variety treated with poultry manure at 5.0×10^3 kg/ha plus NPK at 200 kg/ha. The predominant Carotenoid fraction also trans- β -carotene followed by its 13-Cis-isomer. The former had the concentration 87.89 $\mu\text{g/g}$ (70.96%), while the latter (13-Cis- β -carotene) had 12.36 $\mu\text{g/g}$ (9.80%) concentration. β -cryptoxanthin had 7.32 $\mu\text{g/g}$ (5.84%) concentration. Other fractions include; α -carotene (6.14 $\mu\text{g/g}$) (4.42%), 9-Cis- β -carotene (2.99 $\mu\text{g/g}$) (2.34%), lutein (2.37 $\mu\text{g/g}$) (1.63%) and zeaxanthin (2.03 $\mu\text{g/g}$) (1.38%).

The peak area of trans- β -carotene fraction of this sample appears to be the highest of all the quantified Carotenoid fractions of UMUSPO 3 *Ipomea batatas* varieties under this study. The trans- β -carotene and total β -carotene content of this *Ipomea batatas* sample were 87.45 $\mu\text{g/g}$ and 103.23 $\mu\text{g/g}$ respectively. This was the highest carotenoid yield from all the treatments. Thus the application of poultry manures at 5.0×10^3 kg/ha plus NPK at 200 kg/ha gave the best carotenoid yield for UMUSPO 3 variety.

The chromatogram data of UMUSPO 3 *Ipomea batatas* variety treated with NPK 15:15:15 at 400 kg/ha is shown in Table 7. A total of fourteen carotenoid peaks eluted. The seven identified carotenoids include trans- β -carotene which was predominant with the concentration of 85.67 $\mu\text{g/g}$ (78.30%), followed by β -cryptoxanthin (7.76 $\mu\text{g/g}$) (7.02%); 13-Cis- β -carotene (3.92 $\mu\text{g/g}$) (3.51%), α -carotene 3.28 $\mu\text{g/g}$ (2.70%), zeaxanthin (2.77 $\mu\text{g/g}$) (2.20%), lutein (2.77 $\mu\text{g/g}$) (2.15%) and 9-Cis- β -carotene (1.82 $\mu\text{g/g}$) (1.57%) fractions.

Table 7. Chromatogram data for *Ipomea batatas* (Variety: UMUSPO 3) treated with NPK at 400 kg/ha (VB5)

Peak Number	Retention time (min)	%Area	Peak Number	Retention time (min)	%Area
1	2,130	0,016	8	4,500	7,02
2		0	9	4,923	2,70
3	2,806	0,6	10	5,229	3,51
4	3,113	0,48	11	5,912	78,30
5	3,422	1,22	12	6,625	1,57
6	3,733	2,15	13	7,279	0,08
7	4,240	0,49	14	8,05	0,05

Generally, the peak areas of UMUSPO 3 *Ipomea batatas* variety samples were higher than that of some other *Ipomea batatas* [27] samples showing that UMUSPO 3 OFSP potato samples is a very good source of pro vitamin A carotenoids (especially total β -carotene) (76.76 $\mu\text{g/g}$ – 103.23 $\mu\text{g/g}$) The β -carotene values obtained in this study for the orange-fleshed sweet potato is lower than 218 $\mu\text{g/g}$ in orange-fleshed sweet potato (Brazil cultivars) [22,28,29]. The findings support previous studies on the significant role of orange fleshed sweet potatoes to be utilized as a viable food-based strategy for controlling vitamin A deficiency [30].

The carotenoid concentration of some green leafy vegetables was found to be lower [31] but was significantly improved by cooking. *Pterocarpus mildbreadii* leaf contained a total β -carotene (T β -c) level that was significantly ($p < 0.05$) higher in cooked (212.44 $\mu\text{g/gdwt}$) than in raw leaf (83.53 $\mu\text{g/gdwt}$) [32].

Conclusion

UMUSPO 3 OFSP potato samples is a very good source of pro vitamin A carotenoids (especially total β -carotene). The use of poultry manures at 5.0×10^3 kg/ha plus NPK at 200 kg/ha for soil management will promote carotenoid yield for UMUSPO 3 variety in ultisols of Southeast Nigeria and beyond. Soil nutrient management with Poultry manure at 2.5×10^3 kg/ha + NPK at 200 kg/ha + Agrolyser at 2.7 kg/ha gave the highest yield of beta cryptoxanthin (which provides only half of the amount of vitamin A that can be provided by the beta carotenes).

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