Egg Yolk and Broiler Skin Pigmentation with Sweet Potato Vine Meal[1,2,3]

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ABSTRACT Dehydrated sweet potato vine meal (SPVM) was evaluated as a xanthophyll pigment source for broiler skin and egg yolks. In the experiment with hens SPVM was compared with alfalfa meal in a diet in which these sources provided the only source of pigment. The two diets were formulated to contain the same quantity of pigmenting xanthophylls based on chemical analysis. Egg yolk pigmentation was evaluated by use of the Roche color fan and by chemical analysis. The results indicated that the pigments of this particular sample of SPVM, which was of below average quality, were ingested, absorbed, and deposited in the egg yolk 79% as well as the pigments of alfalfa meal. In the broiler experiment the SPVM as the sole pigment source was evaluated by comparison with standard skin pigmenting substance β-apo-8′-carotenoic acid ethyl ester (apo-ethyl-ester, or AEE). Skin pigmentation was evaluated by chemical analysis of toe web samples. On this basis each gram of dietary SPVM containing 0.417 mg. of total xanthophylls or 0.365 mg. of dihydroxy pigment equivalents per gram was equivalent to 0.276 mg. of AEE. Relative to literature reports of good quality alfalfa meal evaluated under similar conditions the SPVM appeared to be an equally good pigment source for broilers. Chemical analyses of SPVM samples taken at different times during the growing season indicated that there were greater amounts of protein and carotenoids in early season as compared with late season and time of root harvest. It is concluded that the foliage of the sweet potato plant is a potential source of xanthophyll pigments for poultry.

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INTRODUCTION

THE foliage of the sweet potato plant yields approximately one metric ton of dry matter per acre. Current practice is to return this dry matter to the soil. The question arose as to whether or not there was a better use. Chemical analysis revealed that dried sweet potato vines (stems, leaves, and petioles) were relatively high in protein and carotenoids and low in crude fiber. Current poultry feed formulation practice requires the addition of alfalfa meal, corn gluten meal, or some other pigment source to supplement the pigments from the yellow corn in order to provide the high degree of pigmentation of broiler skin and egg yolks expected by the consumer. Consequently, experiments were conducted to evaluate dried, pulverized, sweet potato vines (sweet potato vine meal, SPVM) as a source of xanthophyll for pigmenting egg yolks and broiler skin.

1. Paper Number 4105 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, North Carolina.

2. The use of trade names does not imply endorsement by the North Carolina Agricultural Experiment Station nor criticism of similar products not mentioned.

3. A preliminary report of this work was presented at the 61st annual meeting of the Poultry Science Association, Columbus, Ohio, August, 1972.

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Our values for T_4 half-life are lower than previous reports and are probably closer to the physiological requirements of chickens. Singh *et al.* (1968) found that the thyroxine secretion rate for normal growing chickens was about 2 μg. of T_4 per 100 g. body weight per day. This indicates a thyroid T_4 output of 20 μg. per day for a 1000 g. chicken. The same chicken with approximately 50 ml. of plasma and a plasma T_4 concentration of 3 μg. percent (May *et al.*, 1972) would need to clear the plasma 14 times per day, which suggests a T_4 half-life much less than has been previously accepted.

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NEWS AND NOTES

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TEXAS NOTES

William O. Cawley, Texas Agricultural Extension Service Poultry Specialist, is the author of a new book, "Texas Poultry Federation and Affiliates—A Fifty-Year History."

The 157-page, paper-back volume describes how the Texas Poultry Federation evolved from the Texas Baby Chick Association in the early 1920's. Included is an account of how the need for empty shoe boxes for chick shipping led to the T.B.C.A.'s beginning.

Many of the old timers who struggled to organize and keep the organization going through difficult times are honored in the book: M. Johnson, F. W. Kazmeier, P. E. Payne, D. H. Reid, R. Sherwood, Red Garrison, A. H. Demke, John B. Collier, T. A. Hensarling, J. H. Quisenberry, and J. J. Johnson. There are many pictures, old and new.

The book is available free from the Department of Poultry Science, Texas A and M University, College Station, Texas 77843, or from The Texas Poultry Federation, Box 12946, Capital Station, Austin, Texas 78711.

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MATERIALS AND METHODS

Several varieties of sweet potato vines were obtained at various times during the growing season as indicated in Table 2. The fresh cut foliage was collected in cloth bags which were placed on racks in a drying room. A forced draft of hot air reduced the moisture content to 10% or less within 24 to 36 hours. Dried material was allowed to equilibrate in moisture under ambient conditions for several days before being finely pulverized in a laboratory hammermill. The sample was mixed to insure homogeneity. The resulting material was termed sweet potato vine meal (SPVM). Subsamples were taken for chemical analyses.

Proximate analyses of the SPVM were conducted according to standard methods (A.O.A.C., 1965). Analyses for carotenoids were conducted according to the methods of Purcell (1958) and Purcell and Walter (1968). Tests for the presence of lipoxygenase activity were made by comparing carotenoid content of sweet potato foliage analyzed within one hour after cutting vines with the carotenoid content of the same sample after twenty four hours at room temperature. The same test performed on alfalfa which is known to contain lipoxygenase activity resulted in a substantial reduction in carotenoid content.

A study of egg yolk pigmentation was conducted with White Leghorn hens kept in individual cages. The low pigment basal diet had the following composition in percent: white corn 28.38, soybean meal 26.7, methionine 0.22, vitamin pre-mix 1.4, mineral mixture 2.8, limestone 9.2, cottonseed oil 6.0, a variable amount of corn starch and 10 mg./kg. of N,N'-diphenyl-p-phenylenediamine (DPPD) as an antioxidant. The vitamin and mineral mixes provided sufficient supplemental nutrients to meet the National Research Council (N.R.C., 1971) requirements. Either alfalfa meal or SPVM (sample #7, Table 2) was incorporated into the diet on a weight for weight basis at the expense of corn starch and cellulose (Table 1). the alfalfa meal and SPVM were analyzed for total xanthophylls and incorporated into the diet in amounts sufficient to provide 37.5 mg. of total xanthophylls per kg. of diet. This amount of pigmenting xanthophyll would be expected to produce a desirable degree of egg yolk pigmentation (Scott *et al.*, 1969).

TABLE 1.—*Composition of hen diets in percent*

	Control	+ Alfalfa meal	+ SPVM
Basal diet	74.70	74.70	74.70
Corn starch	21.0	11.3	4.3
Cellulose	4.3	—	—
Alfalfa meal	—	14.0	—
SPVM	—	—	21.0
Total	100.0	100.0	100.0
Protein	18.0	22.0	20.1
Fiber	6.1	5.7	6.1
Xanthophyll, mg./kg.	0	37.5	37.5

Each diet was fed to White Leghorn hens laying eggs at an average rate of 63 percent at the beginning of the experiment. There were 7 or 8 hens per diet. Individual feed consumption and egg production were recorded. Marusich *et al.* (1960) showed that yolk pigmentation is representative of the diet after eleven days of consumption of the diet. Two eggs from each hen were obtained between the thirteenth and seventeenth days of consuming the diet and were evaluated with respect to pigmentation. The eggs were analyzed for the degree of pigmentation by a visual scoring method which utilized the Roche 15 blade color fan.[5] Chemical determination of the xanthophyll content of the egg yolk was conducted according to the Animal Nutrition Research Council (A.N.R.C.) method as described by Scott *et al.* (1968). In this method xanthophyll in a weighed amount of yolk is solublized in a one to one mixture of chloroform and acetone and optical density of the solution is determined at

5 Hoffmann LaRoche, Inc., Nutley, New Jersey.

TABLE 2.—*Composition of sweet potato vine meals and alfalfa meal used in the experiments*[1]

SPVM sample no.	Date	Variety	Crude protein %	Fat %	Crude fiber %	Ash %	Beta carotene mg./kg.	Xanthophyll mg./kg.	DHPE[3] mg./kg.
1.	27 July 1972	Centennial	22.5	3.6	10.2	19.9	218	417	365
	a. After 3 months storage with no preservative						147	306	256
	b. After 3 months storage with ethoxyquin						193	389	345
2.	Sept. 1972	Centennial	12.5	2.7	17.7	11.8	83	163	126
3.	Sept. 1972	Centennial (Washed)	16.6	2.3	20.2	12.4	87	285	235
4.	16 OCT. 1972	FM 57	8.1	1.9	18.7	18.5	68	208	189
5.	13 Oct. 1972	Jewel	12.8	2.0	15.9	13.3	104	285	221
6.	13 Oct. 1972	Centennial	12.9	3.0	16.7	15.8	59	265	202
7.	12 Oct. 1971	Centennial	10.1	3.3	20.4	15.7	76	161	
	Alfalfa meal (Hen exp.)		17.6				117	241	
	Alfalfa meal, dehydrated. (Tabular values)[2]		17.1	2.4	23.0	9.5			

[1] All values expressed on basis of 90% dry matter.
[2] These are tabular values from N.A.S.: Atlas of Nutritional Data on U.S. and Canadian Feeds, 1971.
[3] Dihydroxy pigment equivalents = 1/2 the monohydroxy + all of the dihydroxy carotenoids.

440 nanometers. Results were expressed as milligrams of lutein equivalents per gram of yolk by using an extinction coefficient, $E^{1\%}_{1\,cm.}$ equal to 2590 for lutein (Strain, 1968).

Evaluation of SPVM as a dietary source of xanthophylls for pigmentation of broiler skin was conducted according to the A.N.R.C. method described by Marusich (1970, 1971). In this method the test material is incorporated into a low pigment or pigment free basal diet at levels which will contribute skin pigmenting xanthophyll concentrations up to 22.0 mg./kg. of diet. Up to this level there is reportedly a linear relationship between dietary pigmenting xanthophyll consumption and the quantity of this pigment deposited in the skin of the shank of broilers (Marusich, 1970). Beyond 22 mg. of xanthophyll per kg. of diet and in the range of 28 to 40 mg./kg. which is present in commercial broiler rations (Scott *et al.*, 1969), the skin deposition of xanthophyll bears a non-linear, decreasing relationship to dietary intake and therefore this range of concentrations is not used in assays (Marusich, 1970). A standard skin pigmenting substance, β-apo-8'-carotenoic acid ethyl ester (apo-ethyl-ester or AEE) which was stabilized in the form of gelatin beadlets[6] was fed at levels of 11 and 22 mg./kg. of diet in order to establish a standard response curve from which SPVM could be evaluated. The pigmenting value of SPVM could then be expressed in terms of the reference standard, AEE (Marusich, 1970). Three replicate groups of eight male Pilch DeKalb × Pilch DeKalb chicks per replicate were fed the experimental diets from the eleventh to the thirty-eighth day of age. The chicks were maintained in wire bottom cages in a temperature controlled environment. The basal diet had the following composition in percent: white corn 48.28, soybean meal 32.40, fish meal 3.0, methionine 0.15, vitamin pre-mix 1.12, cottonseed oil 5.0, ethoxyquin[7] 0.0125, mineral mixture 6.50 glucose 3.55. The mineral and vitamin mixtures provided sufficient amounts of these nutrients to meet the N.R.C. (1971) requirements for chicks. The AEE and SPVM were substituted into the diet on a weight for weight basis at the expense of glucose. The SPVM (sample No. 1, Table 1) used in the broiler experiment was protected from xanthophyll loss due to oxidation prior to use in the diets by adding 2.5 percent by weight of coconut oil in which were dissolved 6.7 g. of ethoxyquin/kg. of oil.

Shank pigmentation was evaluated by visual comparison to the 15 blade Roche color fan. The shanks were positioned over a black velvet cloth and illuminated by fluorescent light (two 40 cm. lamps, Sylvania F 15T8-D, daylight) at a distance of 30 cm. Shank color of each bird was scored independently by three observers and averaged to obtain a single value.

Chemical determination of toe web xanthophyll was conducted according to a slight modification of the method of Quackenbush *et al.* (1965). Toe web discs of 6.9 mm. diameter were obtained with a sharpened leather punch. A composite of four toe web discs from each bird for each of the 3 replicate groups were extracted with 100 ml. of a 1:1 (V./V.) mixture of acetone:hexane for 48 hours in the dark at room temperature in the presence of anhydrous sodium sulfate. Solubilized pigment was quantitatively transferred to another flask for removal of solvents under reduced pressure at 50° C. Pigments in the residues were solubilized in 10 ml. of acetone:hexane (2:8, V./V.), transferred to centrifuge tubes, capped and centrifuged to remove insoluble material. Optical density of the supernatant was determined at 450 nanometers. Wave length scans of the toe

6. Obtained from Hoffmann LaRoche, Nutley, N.J.

7. 6-ethoxy-1, 2-dihydro-2, 2, 4-trimethyl quinoline.

web extract from the control, AEE, and SPVM fed chicks showed a broad absorption peak which extended from 440 to 450 nanometers. Optical density values were converted to lutein equivalents by using $E^{1\%}_{1\ cm.}$ equal to 2590 for lutein (Strain, 1938). Results were expressed as μg. lutein/100 sq. cm. of toe web surface. Both dorsal and ventral surfaces are included in the calculation.

RESULTS

Chemical analyses of different samples of SPVM are shown in Table 2. Sample SPVM No. 7 and the commercial preparation of dehydrated alfalfa meal were utilized in the study of egg yolk pigmentation. Sample SPVM No. 1 was used in the broiler pigmentation study. Other samples of SPVM were not used in chick experiments but analyses are presented for comparative purposes. There are differences attributable to stage of growth. Samples early in the season are higher in protein and carotenoids than samples taken at the time of root harvest which occurred in October.

Samples 5 and 6, varieties Jewel and Centennial, were grown at the same location. They are similar in crude protein, fiber, ash, and pigment content. SPVM appears to be as high or higher in pigment content than dehydrated alfalfa meal containing 17% crude protein. SPVM tends to be lower in crude fiber but higher in ash than alfalfa meal. The high ash value may in part be due to adhering soil taken up with the vines upon harvest.

Sample SPVM No. 7 taken in October, 1971 was of very poor quality in terms of protein and carotenoid content. Adverse weather conditions in the weeks prior to harvest resulted in a great loss of leaves. This sample contained a high proportion of stems.

Storage of Sample 1 for 3 months at room temperature without an antioxidant as a preservative resulted in significant loss of carotenoid content. Ethoxyquin greatly reduced the loss from 36.6% to 6.7%.

A fresh cut sample of sweet potato vines was analyzed for carotenoids after 1 hour and again after 24 hours at room temperature. No difference in carotenoid content was evident. Apparently sweet potato foliage contains no lipoxygenase activity. A similar test performed with alfalfa resulted in substantial reduction in carotenoid content.

The objective of the experiment on egg yolk pigmentation was to determine both the quantity and quality of yolk pigmentation. For this reason SPVM was compared with alfalfa meal under conditions in which all of dietary pigment came from one of these sources. The results are shown in Table 3.

There were no differences in egg production. Feed consumption averaged the same for hens consuming diets containing 14% alfalfa meal or 21% SPVM. Yolks of eggs from hens fed the control diet were almost white in appearance. Eggs from hens fed

TABLE 3.—*Egg yolk pigmentation*

	Control	+ Alfalfa	+ SPVM
No. of hens	8	8	7
Egg production[1] (%)	65	61	64
Feed[2] (g./hen/day)	130 ± 11	116 ± 20	115 ± 10
Roche color values[3]	<1	10.0 ± 0.0	9.4 ± 0.3
Lutein equivalents[4]	3.6 ± 0.2	39.9 ± 1.3	31.6 ± 1.3

[1] Eggs/hen/day × 100 between the nineth and seventeenth days of the experiment.
[2] Average feed consumption between the nineth and seventeenth days of the experiment. Mean ± standard error.
[3] Mean ± standard error obtained from visual evaluation with Roche 15 blade color fan. Blades are numbered 1 to 15. Hoffmann-LaRoche, Inc. Nutley, N.J.
[4] See text for explanation. Values are mean ± standard error.

TABLE 4.—*Evaluation of sweet potato vine meal as a broiler skin pigmenter*

	Roche color fan values	Toe web xanthophylls μg. lutein/ 100 sq. cm.	Body weight gain (g.)	Feed/gain
Control	<1	13.5 ± 0.9[2]	949 ± 39[2]	1.87 ± 0.02[2]
SPVM, 3.34% of the feed	2.67 ± 0.11	39.4 ± 1.2	911 ± 7	1.93 ± 0.05
AEE, 11 mg./kg. of feed	3.57 ± 0.39[2]	45.7 ± 2.3	916 ± 48	1.99 ± 0.05
AEE, 22 mg./kg. of feed	5.07 ± 0.31	91.0 ± 2.8	924 ± 11	1.87 ± 0.01

[1]Body weight gain between 11 and 38 days of age.
[2]Mean ± standard error of 3 replicate groups of 8 chicks each.

either alfalfa or SPVM had a color hue that was natural or typical of color expected in an egg yolk. No differences in hue were evident. Intensity of the color as judged by comparison with the Roche color fan was slightly less for eggs from hens fed SPVM. This difference was more evident upon chemical analysis. The difference in lutein concentration between yolks of hens fed alfalfa (39.9 μg./g. of yolk) as compared with SPVM (31.6 μg./g. of yolk) was highly significant ($P < 0.01$). The pigments of this sample of SPVM were utilized only 79% as well as those of alfalfa meal. Variation in xanthophyll content between eggs from the same hen was small compared with variation between hens. This is in agreement with observations of Scott *et al.* (1968).

The objective of the study of broiler skin pigmentation was to evaluate SPVM in terms of the chemically defined, stabilized reference standard, AEE. Results are shown in Table 4. The Roche color fan values are presented for comparison. However, their use in evaluation of an unknown, SPVM, is difficult. The intensity of color represented by each successive blade of the color fan is described by an exponential equation. The form of this equation applicable to shank pigmentation cannot be deduced from our limited data. The data on toe web pigment content produced more definitive results. After correction of toe web pigment values of other treatments by subtracting the amount of pigment in the toe web of the control treatment, an arithmetic plot was made of μg. lutein/100 sq. cm. of toe web surface *vs.* mg. AEE/kg. of diet. A smooth curve drawn from the origin through the points representing 11 and 22 mg. AEE/kg. of diet had an increasing slope. The corrected value for SPVM, 39.4 − 13.5 = 25.9 μg. lutein/100 sq. cm., was applied to the graph and found to be equivalent to 9.2 mg. AEE/kg. of diet. Expressed in terms of AEE, each gram of SPVM containing 0.417 mg. of total xanthophylls was equivalent to 0.276 mg. of AEE; i.e., 9.2 mg. AEE/kg. of diet ÷ 33.4 g. SPVM/kg. of diet = 0.276 mg. AEE/g. SPVM. There were no significant differences in body weight gain or feed conversion among any of the treatments.

DISCUSSION

A review of the literature concerning sweet potatoes revealed a value for xanthophyll content of roots of an unnamed variety, (Scott *et al.*, 1968) and the successful dietary use of dried sweet potato roots (Centennial variety) for pigmentation of egg yolks (Weber, 1969). No information was found concerning use of sweet potato foliage as a dietary source of xanthophyll pigments for poultry.

Results of chemical analyses (Table 2) indicated that samples of SPVM taken at early as compared to late stages of growth were higher in protein and xanthophyll (compare samples 1 and 6, 2 and 4). Proximate analyses reported here with regard to stage of growth

are in agreement with those reported in National Academy of Sciences' publication "Atlas of Nutritional Data on U.S. and Canadian Feeds," 1971.

Alfalfa leaves contain an enzyme, lipoxidase, which is responsible for the rapid degradation of the carotenoids in the cut foliage. Much of the carotenoids will be lost in 24 hours in cut alfalfa allowed to wilt at room temperature. This necessitates rapid drying of foliage in order to stop enzyme action. Sweet potato leaves do not rapidly lose their carotenoid content under conditions in which substantial losses are observed for alfalfa leaves. Presumably sweet potato leaves have no lipoxidase. Consequently the time interval between cuttings and drying of sweet potato foliage is not critical with respect to loss of carotenoids. However, after drying the presence of an antioxidant, ethoxyquin was beneficial in reducing loss of carotenoids during storage.

Results of the experiment on egg pigmentation (Table 3) indicated that xanthophylls of SPVM were digested, absorbed, transported to the ovary and deposited in the yolk. Efficiency of utilization of xanthophyll from SPVM in this experiment was not as good as that from alfalfa meal. However, the quality of this particular sample of SPVM was poor. It contained a high percentage of stems in proportion to leaves, was very high in ash and fiber, and low in xanthophyll.

Results of assay of dietary SPVM as a pigmenter of broiler skin (Table 4) indicated that 1 gram SPVM containing 417 mg. of total xanthophylls per kg. of SPVM was equivalent to 0.276 mg. of AEE. The value of SPVM relative to alfalfa or corn gluten meal can be approximated from comparison of results in this assay with results of assays reported by Marusich (1971). He computed the dihydroxy pigment equivalents (DHPE) content of alfalfa meal and corn gluten meal by summing all dihydroxy pigments plus 1/2 of the monohydroxy pigments. The resulting value is a good indicator of the content of xanthophyll pigments which contribute to skin pigmentation in the broiler. On this basis our sample of SPVM contained 365 mg. of DHPE/kg. When fed as 3.34% of the diet in our experiment SPVM appeared to give as good a degree of skin pigmentation as diet containing 3.55% dehydrated alfalfa meal (310 mg. DHPE/kg. of meal) or 3.60% corn gluten meal (306 mg. DHPE/kg.) fed in experiments reported by Marusich (1971).

Results of our experiments indicated that xanthophyll pigments in sweet potato vines were good pigmenters of both egg yolk and broiler skin. Therefore sweet potato vine meal is a potential source of pigment.

Conditions required to obtain high quality SPVM for use in poultry feeds need to be determined. Consideration must be given to plant variety, fertilizer application, and time of vine harvest. Harvesting machinery and the time from cutting to drying are additional considerations. Drying and processing equipment could be the same as used for alfalfa meal.

Major sites of sweet potato production occur in North Carolina and Louisiana. The large yield of sweet potato foliage relative to the geographical area of production may make possible profitable installation of drying and processing equipment in order to obtain the above ground portion of the crop. Production of SPVM would probably occur only in the fall of the year and would slightly precede or coincide with harvest of the roots. Sweet potatoes are annual plants which are propagated each spring from rootings. A possibility may exist for the development of a sweet potato cropping system to be used solely for the production of SPVM with no intent of harvesting the roots. Preliminary studies have indicated that sweet potato vines may be repeatedly trimmed to yield a high protein, high xanthophyll SPVM; however, the yield of roots was markedly diminished under those conditions.

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NEWS AND NOTES

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XV WORLD'S POULTRY CONGRESS

An unusual selection of scientific and industrial tours have been arranged for participants in the XV World's Poultry Congress which will be held in New Orleans, Louisiana, August 11-16, 1974.

The tours feature visits to the nation's foremost egg and poultry operations, research centers, university science and food departments, the U.S. Department of Agriculture in Washington, D.C., and the Chicago Mercantile Exchange. Planned are open discussions with industry leaders, consultations with government scientists and administrators, and meetings with genetics experts and management consultants. Breeders, producers, processors, and feed companies are all cooperating to give tour members first-hand observations in every area of the industry.

With sightseeing and hosted entertainment in the principal cities on the itineraries, these tours scheduled prior to and following the XV World's Poultry Congress will enable members to combine the values of an educational experience with the pleasure of a vacation holiday. Each of the four tour offerings will be escorted by a qualified tour leader. Tour costs give participants the advantage of lower rates available through group travel.

Detailed information on the World's Poultry Congress Scientific and Industrial Tours is available from Mr. Richard Sykes, Courtesy Travel Service, 1629 K Street, N.W., Washington, D.C. 20006.

A.A.A.P. NOTES

Dr. J. T. Tumlin was recently elected President of the American Association of Avian Pathologists. He is Director of Technical and Professional Services, Vineland Laboratories, Inc., a subsidiary of Damon Corporation.

The Association was formed in 1957 to promote the mutual interest of individuals engaged in the field

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