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Potential of Annatto in Agroindustries and Animal Feed: Fragrance, Flavor, Taste and Color of *Bixa orellana* L. Derivatives

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Abstract: Experiments were conducted to assess the level of dye expressed as bixin in annatto seed full of germplasm bank of the Experimental Farm of Pindorama IAC and its application in obtaining animal products, the use of pigmented ground whole seed in the diet of laying hens. The content of total carotenoids expressed as bixin in the collection of seeds ranged between 2.98% to 5.91%. The seeds used in chicken feed had a concentration of total carotenoids expressed as bixin 3.12 ± 0.05 mg/100g. Assessments of production parameters, physical assessment of the color range by colorimetric spectrophotometer, and the axial Minolta L * C * h showed satisfactory means of production and increasing levels of pigmentation of the buds when compared to control.

Key words: new nutrients, pigmentation, phytonutrient, annatto

INTRODUCTION

The varieties of plants of biodiversity with sustainable potential for use as dyes has been the option to find support legislation and meet the growing demand on the brink of global trend for natural products healthy. The remarkable preference for natural dyes is given increase in consumption of dyes extracted from plants and in particular those derived from annatto.

Among the plants considered medicinal and aromatic herbs, annatto seeds, stand out by having multiple functions. The annatto plant Bixácea family, genus *Bixa orellana* (Linnaeus), native to tropical America and is an elective in a sustainable, was first described in Brazil according to the memoirs of the expedition led by Francisco Orellana in the Amazon River about the year 1541.

Plant hardy shrub and perennial plant with potential dye presenting nutritional value in the seed whose chemical composition has besides the dye in arils, essential oils, fiber and resin with Nutraceutical and functional value. The fruit called of bunch of annatto provide 20 to 60 seeds and from the fourth year of production can reach 1.600 kg to 2.000 kg/ha, and productivity and carotenoids arils cultivar dependent[9]. The content of dye in the pericarp of the seed is highly variable depending mainly on the variety of plant, soil, climate, humidity, cultural and time of year of production.

According to the concept of use as additives and spices, annatto extract is rich in medicinal compounds and pigments, such as geranilgeraniol, bixin and norbixin and tocotrienols[20]. Bixin and norbixin have solubility in oil and salt norbixin, obtained after alkaline hydrolysis, is soluble in water [11]. Depending on the concentration in solution form the colors ranging from yellow orange to reddish orange.

Although the chemical composition of the seed, Alonso[1] reported in the Treaty of herbal medicine the following compounds: carotenoids provitamin A and b-carotene and cryptoxanthin, bixin between 1 and 6% on dry matter, other carotenoids such as lutein, zeaxanthin and orelina; tocopherols and tocotrienols in the essential oil, proteins between 13 and 17%, considerably higher concentration of amino acids lysine and tryptophan and to a lesser methionine, isoleucine, leucine, phenylalanine, threonine, 5% fat, 5.4% ash, and high levels phosphorus.

Galindo-Cuspineira *et al*.[10] by GC-MS reported the presence of 107 compounds, and some oil and other soluble in water. In the study, the authors identify and quantify aromatic compounds responsible for the characteristic fragrance. Identified in the aqueous and oily the following compounds: alcohols, aldehydes, alkanes, alkenes, ketones, esters and acids,

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heterocyclic compounds, monoterpenes, sesquiterpenes. Carotenoids bixin and norbixin responsible for the color, and the lowest level in a-and b-carotene[4,8,20]. There is evidence that compounds like geranilgeraniol, tocotrienols and carotenoids in the seed annatto may be beneficial to health, acting in the prevention of atherosclerosis and hepatic lesions[7,15,19]. Addition to contributing to the flavor and aroma, volatile compounds found in the extract of annatto, as monoterpenes (geraniol, linalool) and sesquiterpenes, have antioxidant, antimicrobial and anticancer.

In Europe, the yellow-red color has been used in butter, margarine and cheese. Annatto extract can also be used as pigments and flavoring in confectionery flour and sugar, meat products, beverages and snacks. The dye derived from annatto has numerous other applications in the food industry in sausages, cheese, yogurt, ice cream, butter, crackers, margarines, liqueurs, spice, extruded, dairy beverages, bakery products, beverages, powdered mixes and bakery items. The aroma, flavor and astringency typical of fresh seed has attracted fans of cooking.

It is known there are two germplasm banks active in production. Bank of EMEPA in Paraíba[9] and another at the Experimental Farm of the Agronomic Institute (IAC) of Pindorama-SP[3].

1. Experiment 1: Validation of analytical methodology to determine total carotenoids expressed as bixin in annatto seed and characterization of germplasm collection Pindorama - SP.

1.1. Validation of a methodology for analysis of total carotenoids expressed as bixin in annatto seeds

1.1.1.Introduction: The annatto (Bixa orellana L.) is a shrub native to tropical America, whose seeds are widely used in industrial scale production of dye. The majority of the carotenoid annatto seeds is bixin representing at least 80% of carotenoids. Industrially are obtained two types of dyes: the soluble form of the bixin and norbixin and water soluble as the sodium salt of norbixin. Methods to quantify bixin commonly employ organic solvents such as acetone, chloroform, ethanol, methanol or solutions of sodium or potassium, with the use of temperature or not, in whole or ground seeds. This study aimed to validate a method to quantify the total carotenoids expressed as bixin and/or salt norbixin in annatto seeds with hot saponification.

MATERIAL AND METHODS

For the analysis about 10g of sample was mixed with 60 mL of soap solution (290ml of castor oil, 45% KOH 100mL and 190ml water. Heat until the solution becomes clear. Dilute 290ml of this solution in 420mL of 45% KOH and 3, 2 liters of water), heated and kept boiling for a minute. The mixture was cooled, the mass adjusted for 250g with deionized water, agitated for 2 minutes and proceeded dilutions for spectrophotometric reading. The reading was done at 453 nm was used and the extinction coefficient of 2850. The validation were included the following criteria: specificity, linearity, sensitivity, precision and robustness. In the test of robustness were evaluated: the wavelength and extinction coefficient, the sample taking, analysis of initial weight or volume, shaking time and after saponification time boil.

RESULTS AND DISCUSSION

The method is specific, showed good linearity with correlation coefficient of 0.9996 for the full estimated between 7.4 to 49.6 mg/mL, the limit of detection was found to be 0.31 mg/100 g and the limit of quantification of 0.63mg/100g. The method showed good precision with a coefficient of variation of 2% to the level of dye usually found in the seed.

In the test of robustness significant difference was observed for sample taking, shaking time, initial volume of 250mL, and reading the 482nm extinction coefficient of 2870.

The calculation of uncertainty were considered the uncertainties of mass, volumetric glassware, flask the pipettes, spectrophotometer, the extinction coefficient and of the method as shown in Figure 1. To determine the expanded uncertainty was used for the extinction coverage factor (K) equal to 2, with 95% [13].

The validated method showed a concentration of total carotenoids expressed as bixin, equal to 5.46 g/100g seed and an expanded uncertainty of ± 0.30 g. The results in grams of total carotenoids expressed as norbixin salt per 100g of seeds was 4.71 with an expanded uncertainty equal to ± 0.26. For both cases we used a coverage factor (K) equal to 2.

Conclusion: The validated method proved to be specific with good linearity in the range evaluated, detection limit of 0.31 mg/100g and quantification limit of 0.63 mg/100g, coefficient of variation of 2% between the replicates and an expanded uncertainty for process of validation of 5.50%. The uncertainty of the method showed the highest relative contribution to the expanded uncertainty and was around 30% and the spectrophotometer contributed the smallest share, about 2%.

1.2 Characterization of the germplasm bank of Pindorama - SP, on the levels of carotenoids

1.2.1.Introduction: The evolution of the annatto (*Bixa orellana*, L.) in Brazil over the last ten years is remarkable. The work carried out in different niches of

knowledge about this culture and its technology have produced an accumulation of knowledge that allows today to find highly productive plants and seeds containing a high concentration of carotenoids. While in the recent past to find seeds with levels of carotenoids than 3% was a rare event, now values greater than 4% has become a routine. However, more knowledge of this culture has also been shown that these seeds are not just a depository of carotenoid pigments, they contain a number of other highly interesting substances, such as geranilgeraniol and tocotrienols, as well as being a source of protein and carbohydrates for feed. Therefore, studies must continue and the existing germplasm banks play a fundamental role in this process. One of the best collections of annatto in Brazil is located in the APTA Regional Centro Norte, located in the municipality of Pindorama of the São Paulo State in southeastern Brazil. The introduction of these plants was initiated in 1988 and since then has been the subject of several studies. This study aimed to identify the carotenoids content of seeds of plants that make up this collection, with a validated methodology.

1.2.2. **Materials and Methods:** Plants that make up the collection of Pindorama were identified by numbers and the characterization of the levels of total carotenoids expressed as bixin was performed as described by Silva [19]. Measurements were conducted in triplicate analysis.

1.2.3. **Results and Discussion:** The results ranged from a minimum of 2.98 ± 0.06 g/100g (sample 6) to a maximum of 5.91 ± 0.06 g/100g (sample 34). Most of the samples has fluctuated in the range of 3 to 4g/100g (samples 1, 2, 3, 4, 9, 10, 11, 12, 13, 15, 16, 19, 21, 23, 24, 26 and 28). The amostras14, 17, 31 and 32 showed levels of bixin in the range between 4 and 5g/100g and samples 18, 22, 27 and 34 showed levels of bixin 5g/100g above (Table 1, Figure 2).

1.2.4. **Conclusion:** Considering the total lack of cultural practices in the collection at the time of seed harvest. the results obtained in the samples available were very good, with averages of bixin in the collection ranging between 2.98 and 5.91 g/100g seed.

2. Experiment with livestock: 2.1 Experiment 2 - Addition of carotenoid compounds derived from seed full ground annatto (Bixa orellana L.) IAC type in the diet of laying hens in egg production special

2.1.1 General Remarks and Introduction: The implementation of strategies to improve the nutritional composition and quality of food and animal products has recently emerged as an interface animal scienc, food science and human nutrition. This approach has been used to change the composition of products in order to be more consistent with the nutritional standards of the human diet. The egg is considered an excellent source of food. Graciously, our culinary has adopted the egg to be a complete food in nutrients and high consumer acceptance. From the nutritional point of view, the egg is considered ideal target for dietary modification leading to the development of food with functional or nutraceutical properties.

When considering the benefits of improving the quality of eggs in the concentration of various nutrients, especially compounds carotenoids, various studies have been conducted in order to strengthen eggs[12]. The food industry has envisioned increasing expansion of products and egg by-products. Alternatively, the oil-yolk eggs, diet modification layer, can be rich source of natural antioxidant compounds based on carotenoids.

The strategies to meet the increasing demands of natural dyes in the face of restrictions those artificial pigments demonstrated the potential risk to public health have been anchored in decisions of the Committee of Experts of the FAO/WHO[14] to detect experimentally some degree of the various toxicity dyes in use.

2.1.2 **Material and Methods:** The polls were conducted October 2007 to January 2008 in the experimental bird vivarium of the Laboratory for Animal Health. Bauru and CCQA of ITAL. institutions of APTA Department of Agriculture of the Sao Paulo State. This study used a completely randomized design. using 120 hens Label Rouge with 32 weeks old, marked in five treatments with three replicates of eight birds. Ration was provided ad libitum in trough type feeder and water fountain nipple. Diets were isocaloric and isonitrogenous and were formulated according to NRC[17]. The diets of the treatments were composed of a basal diet of white maize (MB) and soybean, the treatments from two to five diets of white corn, the hens have added levels of 1.00%; 1.25%; 1.50% and 1.75% of ground annatto seed (SMU) in the diet (Table 2). The analysis of the content of bixin SMU was performed following the method of Carvalho *et al*.[5]. Eggs used for the determination of objective color of fresh buds were harvested in the ninth week. To obtain the color by color fan Roche yolk color fan (LC) were sampled twelve eggs per treatment and after broken, the contents were exposed uniformly in a petri dish in an environment with fluorescent lighting and the score of yolk color was obtained by individual four trained evaluators. We used portable spectrophotometer CM 508D - MINOLTA and CIELAB system, illuminant

D65. illumination angle 10. with results expressed in the axial coordinate L * (lightness). C * (chromaticity) and h (hue). Statistical analysis used the SAS[18] and for comparisons between the means was applied Tukey test, adopting the level of 5%.

2.1.3 **Results and Discussion:** The seeds used had a concentration of total carotenoids expressed as bixin. equal to 3.12 ± 0.05 mg. Treatments supplemented with levels of 1.00% to 1.50% in the diet of the SMU-based DM did not influence the consumption of hens compared to an average of 108.67 g/hen/day in control group (T1). except reduction in average 99.50 g/hen/day consumption of T5. Production parameters kg feed/kg egg, kg feed/dozen egg laying rate and were not influenced by treatments, except the mean of 1.60 (kg/dz) and 85.30% (IP) in group 5 showed decreases significant compared to other treatments (Table 2). The parameters of internal and external quality of the egg were not affected by treatments in agreement with that reported by Arraya *et al*.[2] who reported the use of 1.06% of full seed in the diet of laying hens in order to increase the pigmentation of egg yolks.

Yolk color evaluated by LC medium showed increased (P<0.05) to 8.25 (T2) to 11.75 (T5) between contrasting treatments (P<0.05) with a unit average obtained in control (T1). They stressed the efficiency of carotenoid-derived oil on egg yolk color (Table 2). The analysis of objective color of yolk fresh in L * C * h showed evidence (P<0.05) among the treatments. The average color of the gem objective in nature in axial coordinates L * C * h varied (P <0.05) to 16.59% lower in L * (T1= 64.44 to T5 = 53.75) tend to pale the saturation of the color yellow. the higher 200.41% in C * (T1 = 12.09 to T5 = 36.42) with accentuation of the yellow-orange and. most 186.18% in h (T1 = - 78.78 to T5 = 67.89) with a more intense yellow, confirming the potential of annatto carotenoids in egg yolks and fortified increases (P <0.05) increased significantly in color. via transfer of pigments from the diet to the egg.

The regression equations predicted that: L * yolk decreased linearly (Y = 0.2015 X + 64.5240, R2 = 0.93) with increasing consumption of pigments of annatto in the diet, C * increased linearly in the yolk (Y = 0.4875 X + 12.4830, R2 = 0.95) in proportion to the increase in consumption of pigment in the feed, h: showed accentuation of the yellow hue (cubic regression: Y = -0.0002 x3 - 0.0736 X2 + 7.1667X - 78.78, R2 = 0.99) yolk to limit intake of approximately 45 mg/hen/day of pigment present in 1.25% of SMU (Figure 3). The results of this study projected the magnitude of incorporation of the carotenoid annatto from diet to yolk, confirming reports of Marusich and Bauernfeind[16] to mention that the complex micelles of fatty acids and carotenoids formed in the light of the small intestine by the action of digestive juices and bile salts and are absorbed by passive diffusion. Chylomicrons in the enterocytes containing esters of carotenoids are transported by the lymphatic ducts to the liver. By intense hormonal action of estrogens in hepatocytes or ovary after resynthesized and conjugated lipoproteins, carotenoids are carried to the target tissue and specific receptors are incorporated into the oocytes of the hen. According to the authors above, the diet lacking carotenoids your pet may be predisposed to oxidative stress of tissues in view of numerous biochemical functions they play in the animal organism. Abundant reserves of these compounds in tissues and products (yolk) are protective mechanisms and would supply higher demands in certain physiological states and embryo production[6].

Table 1: Levels of totals carotenoids expressed as bixin in the annatto seeds collection Pindorama-SP

Samples (nº)												
6	12	13	15	16	19	28	26	23	11	4	3	21
Totals carotenoids (Bixin) (g/100g)												
2.98^{a} ± 0.06	3.10^{ab} ± 0.04	3.10^{ab} ± 0.04	3.10^{ab} ± 0.09	3.21^{abc} ± 0.09	3.38^{bcd} ± 0.07	3.47^{cde} ± 0.02	3.48^{cde} ± 0.04	3.56^{def} ± 0.14	3.57^{def} ± 0.07	3.58^{def} ± 0.06	3.61^{def} ± 0.23	3.68^{efg} ± 0.12
Samples (nº)												
9	24	1	2	32	14	29	17	31	22	18	27	34
Totals carotenoids (Bixin) (g/100g)												
3.77^{fg} ± 0.09	3.84^{fgh} ± 0.13	3.93^{gh} ± 0.07	3.93^{gh} ± 0.07	4.08^{hi} ± 0.12	4.24^{ij} ± 0.10	4.26^{ij} ± 0.09	4.48^{jk} ± 0.10	4.69^{k} ± 0.08	5.02^{l} ± 0.08	5.18^{lm} ± 0.02	5.29^{m} ± 0.04	5.91^{n} ± 0.06

Means with different letters in rows differ (P<0.05) by Tukey test.

Table 2: Means consumption (g/hen/day), feed conversion (kg feed/kg egg and kg feed/dozen eggs), Haugh unit (HU), specific gravity (SG), egg weight (g), posture index weight (I.P), thickness (EC) and shell percentage (%), weight and percentage of albumen (%) and yolk (%) and objective color of raw egg yolk in L * C * h in egg eggs from hens

Treatments	T1	T2	T3	T4	T5
SMU ration(%)	0	1.00	1.25	1.50	1.75
		Annatto carotenoids in the diet (mg/hen/day)			
	0.00^{D}	35.78^{C}	43.51^{B}	50.73^{A}	50.91^{A}
Parameters		Productive performance and egg quality [1]			
g/hens/day	108.67^{A}	106.00^{A}	104.70^{A}	104.30^{A}	99.50^{B}
Kg rat./kg egg	2.06^{A}	2.01^{A}	2.08^{A}	1.99^{A}	2.10^{A}
Kg rat./doz.egg	1.43^{A}	1.50^{A}	1.55^{A}	1.51^{A}	1.62^{B}
U.H	90.25^{B}	98.58^{A}	97.72^{A}	95.30^{A}	96.80^{A}
G.E (g/L)	1.092^{A}	1.092^{A}	1.090^{A}	1.091^{A}	1.093^{A}
Egg weight (g)	61.92^{A}	59.85^{A}	60.10^{A}	59.98^{A}	59.79^{A}
I.P (%)	91.07^{A}	87.90^{A}	88.50^{A}	89.55^{A}	85.30^{B}
E.C (mm)	0.3963^{A}	0.3982^{A}	0.3817^{A}	0.3899^{A}	0.3895^{A}
P.C (g)	6.38^{A}	6.63^{A}	6.52^{A}	6.24^{A}	6.40^{A}
Egg shell (%)	10.30^{A}	10.93^{A}	10.28^{A}	10.05^{A}	10.41^{A}
Albumen (g)	39.17^{A}	37.93^{A}	38.90^{A}	39.50^{A}	39.83^{A}
Albumen (%)	63.23^{A}	62.31^{A}	62.90^{A}	62.67^{A}	62.79^{A}
Yolk (g)	16.38^{A}	17.68^{A}	17.01^{A}	17.10^{A}	17.30^{A}
Yolk (%)	26.46^{A}	28.90^{A}	26.79^{A}	27.98^{A}	26.97^{A}
Color score [2]	1.00^{D}	8.25^{C}	8.75^{CB}	10.25^{B}	11.75^{A}
		Objective color of raw egg yolk in the axial L*C*h [3]			
L*	64.44^{A} ± 0.88	56.58^{AB} ± 2.43	57.85^{AB} ± 5.54	53.54^{B} ± 0.81	53.75^{B} ± 3.35
C*	12.09^{B} ± 0.38	32.87^{A} ± 4.40	30.33 ^{A} ± 6.31	39.00^{A} ± 4.81	36.32^{A} ± 5.17
h	-78.78^{C} ± 1.20	74.13^{A} ± 1.92	76.96 ^{A} ± 6.90	69.61^{AB} ± 3.17	67.89^{B} ± 4.04

Chemical composition determined of SMU (%)[4]

D.M	P.B	F.B	E.E	M.M	BIXIN	N.D.T	E.Ñ.N
92.43	14.27	13.84	3.35	5.25	2.44	71.91	63.29[1]

[1]Means with different letters in the row differ (P<0.05) significantly by Tukey test. 2 Fan colorimetric Roche yolk color fan. [3]L * C * h: Mean ± standard deviation. [4]DM = Dry matter; CP = Crude protein; FB = crude fiber; EE = Ether extract; MM = mineral matter; TDN = totals digestible nutrients (estimated); NFE = nitrogen free extract and the gross energy determined in SMU = 4496. 97 cal/g.

Means with different letters in the row differ (P <0.05) significantly by Tukey test. 2 Fan colorimetric Roche yolk color fan. 3 L * C * h: Mean ± standard deviation. 4 MS = dry matter. CP = Crude protein; FB = crude fiber. EE = Ether extract. MM = mineral matter. TDN = total digestible nutrients (estimated); NFE = nitrogen free extract. the gross energy determined in SMU = 4496. 97 cal / g.

Conclusion: Additional levels of carotenoids from annatto in the diet of laying hens stood out by increased of the yolk eggs pigmentation. The parameters of external and internal quality of eggs were significantly influenced in the context of better quality and color attributes of enriched eggs and special.

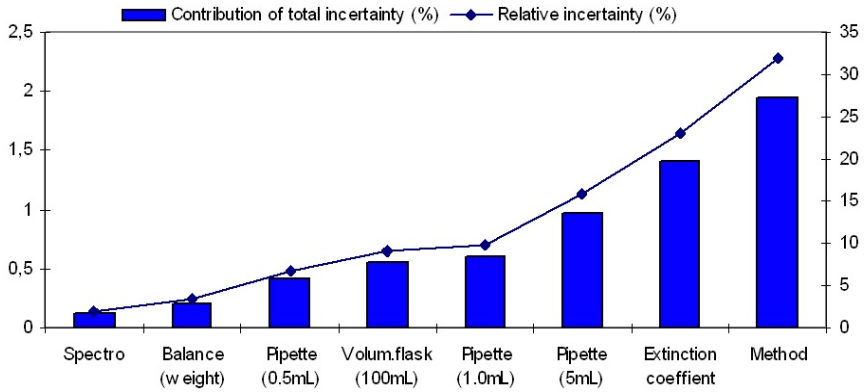

Fig. 1: Uncertainties histogram

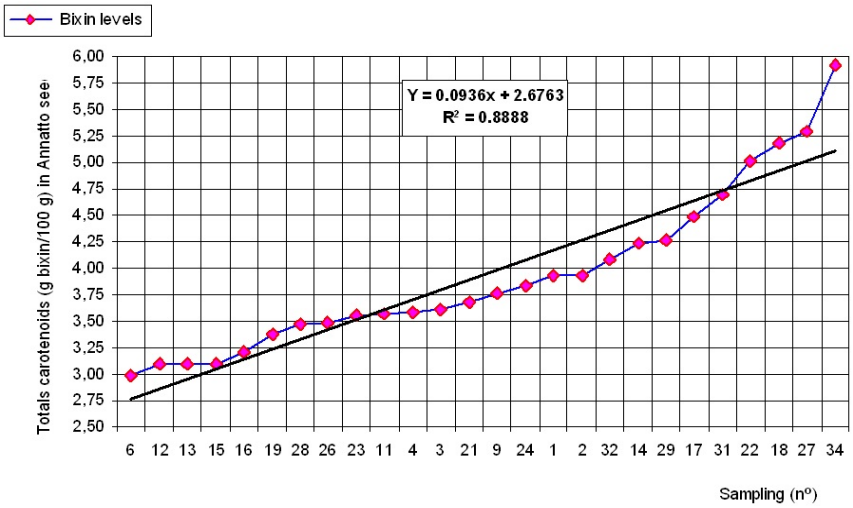

Fig. 2: Distribution of average total carotenoids expressed as bixin in seed (g/100g) of the germplasm bank of the Experimental farm of the IAC Pindorama (Sao Paulo, 2008) predicted by the equation Y = 0.0936 X + 2.6763

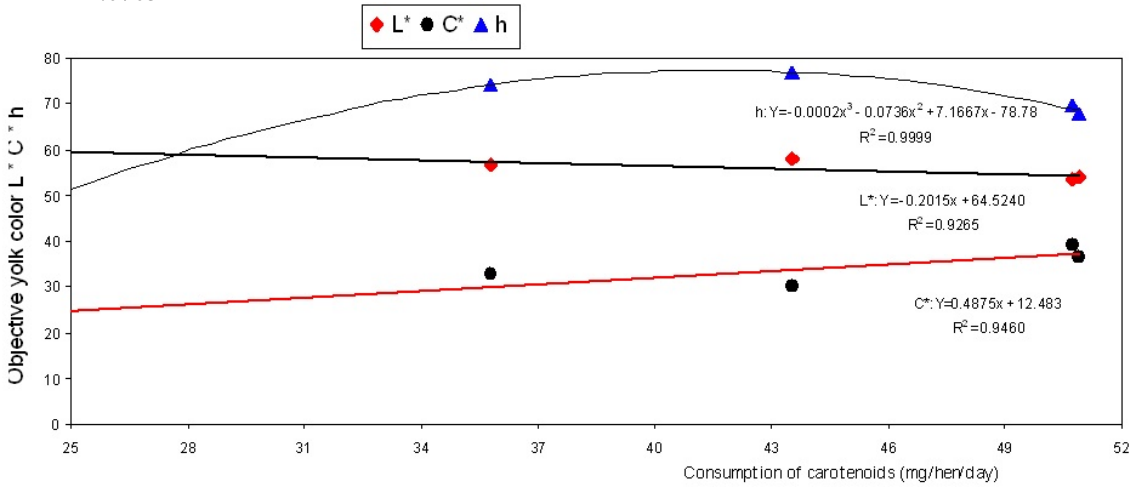

Fig. 3: Variation of the average color of the gem objective L * C * h according to the concentrations and consumption of carotenoids from annatto the treatments studied

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