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Plasma fatty acid profile in dairy cows associated with the inclusion of annatto in their diet

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ABASTRACT. The objective of this study was to evaluate the plasma lipid profile and plasma fatty acids of dairy cows receiving diets supplemented with annatto. A total of 32 Holstein cows (550 kg), distributed in a completely randomized design, were allocated to individual stalls and submitted to following treatments: C0 = no annatto; C4 = inclusion of annatto at 4 g kg^{-1} dry matter (DM) of diet (0.07 g bixin kg^{-1} diet); C5 = inclusion of annatto at 5 g kg^{-1} DM of diet (0.09 g bixin kg^{-1} diet); and C7 = inclusion of annatto at 7 g kg^{-1} DM of diet (0.12 g bixin kg^{-1} diet). Blood samples were collected via epigastric vein puncture, centrifuged, and frozen for subsequent analysis. The results indicate that the inclusion ($p > 0.05$) of annatto does not decrease the total cholesterol or low and high density lipoproteins. However, it impacts the profile of fatty acids, evidenced by the reduction ($p < 0.05$) in levels of hypercholesterolemic fatty acids viz, myristic acid and palmitic acid. It also causes an increase in the levels of arachidonic acid, rumenic acid, linoleic acid, and total polyunsaturated fatty acids. Therefore, bixin included in the diets of dairy cows induces changes in the plasma fatty acid profile.

Keywords: antioxidant; bixin; cholesterol; linoleic acid; polyunsaturated fatty acids.

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Introduction

Cardiovascular diseases are among the leading causes of deaths in the world. The negative effects of a diet rich in cholesterol and saturated fats and its close relationship with the development of such diseases has been evidenced (Garaffo et al., 2001). In order to minimize the effects of hypercholesterolemia, interferences of dietary origin, viz., ingestion of compounds with antioxidant properties, have been proposed to decrease the concentration of circulating cholesterol (Portela, Neto, Silva, Simões, & Almeida, 2014).

In the food industry, carotenoids are often used as natural dyes to attract consumer attention (Sigurdson, Tang, & Giusti, 2017). However, their function can be referenced in a more noble, beneficial, and functional way to health (López-Vargas, Fernández-López, Pérez-Álvarez, & Viuda-Martos, 2013). Among other properties, carotenoids possess antioxidant and anticancerous functions, and prevent the occurrence of non-transmissible chronic diseases (Carocho, Morales, & Ferreira, 2018; Derakhshan et al., 2018; Jiang & Xiong, 2016). Several carotenoids can be found in nature, some with recognized activities. Bixin, found in annatto that is extracted from seeds of the achiote tree (*Bixa orellana* L.) native to Tropical America (Nathan, Rani, Rathinasamy, & Dhiraviam, 2019; Van Cuong & Chin, 2016; Yong et al., 2013), stands out in this group. Annatto is an important raw material used in the Brazilian food industry, especially in the production of sausage, pasta, cheese, ice cream, and confectionery. Annatto corresponds to approximately 70% of the dye market worldwide and 90% of the natural dyes used in the Brazilian food industry (Lemos et al., 2011).

Bixin possesses bioactive functions, owing to its antioxidant activity. More specifically, it regulates the production of reactive oxygen and nitrogen species, and thus prevents diseases. (Patnaik, Mishra, Choudhary, Panda, & Behera, 2011). Bixin also exhibits hypolipidemic effects (Ferreira et al., 2013).

The objective of this study was to evaluate the biochemical parameters and plasma fatty acid profile of dairy cows fed diets supplemented with bixin.

Material and methods

The experiment was carried out at the Experimental Station of São Bento do Una - PE, belonging to the Agronomic Institute of Pernambuco – IPA, located in the mesoregion of the Northern Agreste and micro region of the of Vale do Ipojuca, at 8° 31'16" south latitude, 36° 33' 0" west longitude, and 650 m altitude. The average precipitation of the region is 601.6 mm per year, concentrated from March to July, which corresponds to approximately 60% of the total annual volume. The analyses were carried out at the Animal Nutrition Laboratory of the Federal Rural University of Pernambuco – Garanhuns Academic Unit.

A total of 32 lactating dairy cows (± 85 days in lactation) with mean body weight of 550 ± 70 kg and average yield of 20 ± 7 kg of milk per day were used. Eight animals per treatment were allocated to individual stalls. The diet comprised sugarcane silage, forage palm (*Opuntia ficus-indica* (L.) Mill) and commercial concentrate of corn and soybean. The diet with annatto (Table 1) was prepared according to the Nutritional Requirement of Dairy Cattle (National Research Council [NRC], 2001) for lactating cows, with an average 12% crude protein and 4% ether extract. The amount of diet offered was 2.5% of the body weight, divided in two portions daily in the morning (40% of the total diet plus annatto), and in the afternoon (60% of the total diet).

A randomized experimental design was used, consisting of four diets that characterized the treatments: C0 = no annatto; C4 = dietary inclusion of annatto at 4 g kg^{-1} DM (0.07 g bixin kg^{-1} diet); C5 = dietary inclusion of annatto at 5 g kg^{-1} DM (0.09 g bixin kg^{-1} diet); and C7 = dietary inclusion of annatto at 7 g kg^{-1} DM (0.12 g bixin kg^{-1} diet). The food color based on annatto was purchased from the Ouro Verde factory, located in the region of Garanhuns, and was supplied to animals once a day directly in the feeding trough.

Table 1. Ingredients and chemical composition of experimental diets.

Composition	Experimental Diets			
	C0	C4	C5	C7
Ingredients (g kg^{-1} DM)	361.6	427.1	375.6	396.8
Concentrate	482.2	428.3	467.8	450.1
Silage from sugarcane	156.2	140.5	151.6	146.4
Forage palm	0.0	4.0	5.0	7.0
Annatto	361.6	427.1	375.6	396.8
Chemical Composition (g kg^{-1} DM)				
Dry matter	307.1	329.9	313.2	323.6
Crude protein	114.3	129.2	117.4	122.2
Neutral detergent fiber	445.6	421.7	438.3	430.3
Ether extract	40.8	42.7	41.1	41.7

C0 = no annatto; C4 = dietary inclusion of annatto at 4 g kg^{-1} DM (0.07 bixin g kg^{-1} diet); C5 = dietary inclusion of annatto at 5 g kg^{-1} DM (0.09 g bixin kg^{-1} diet) and C7 = dietary inclusion of annatto at 7 g kg^{-1} DM (0.12 g bixin kg $diet^{-1}$)

The bixin in annatto was quantified according to the protocol of Yabiku and Takahashi (1992). The concentration of bixin was determined by the equation:

$$\text{Bi}(\%) = \frac{\text{Ab} \times \text{Df} \times \text{V}}{(\text{m} \times \text{S})} \times 1.037$$

where:
Ab = absorbance;
Df = dilution factor;
V = initial sample volume;
S = specific coefficient of extinction;
M = sample mass;
1.037 = conversion factor.

Cows were allocated to individual 36 m^2 stalls, with free access to feeders and drinking troughs. The experimental duration was 51 days per animal, of which the first 21 were for acclimatization. Blood samples were collected on the 30th day in a 40 mL vacutainer tube containing EDTA K3 via puncture of the epigastric vein after milking in the morning. Blood was centrifuged at 3200 rpm for 20 min, and the plasma was separated and stored at -20°C for future analysis.

For the determination of oxidized low density lipoprotein (LDL), total cholesterol (TC), and high density lipoprotein (HDL), colorimetric commercial kits of the brands Elisa MBS738313, Biotechnology Life, and

Labtest Ref. 13, were used respectively. The value of LDL was estimated by calculating the difference between the values of total cholesterol and HDL

The lipid fraction of the blood was extracted according to the protocol proposed by Folch, Lees, and Sloane-Stanley (1957) to determine the fatty acid profile, using aliquots of 3 mL of plasma, 20 mL of chloroform and methanol (2:1) solution, and 4.4 mL of 1.5% NaCl solution. After that, samples were centrifuged at 2400 rpm for 10 min at 22°C, following which 300-350 mg of fat was collected and subsequent methylation was performed in two steps, the first with 3 mL of 10% HCl and, the second using1 mL of hexane and 10 mL of 6% K_2CO_3. Next, samples were centrifuged at 500 ×*g* for 5 min for layer separation (Kramer et al., 1997). The nonpolar layer was transferred to a 13 × 100 mm tube containing 1 g of Na_2SO_4 and activated charcoal (1:1), and the tube sealed with a teflon screw cap, centrifuged, and transferred to an Eppendorf flask for analysis in a gas chromatography apparatus.

A Shimadzu 14B gas chromatograph equipped with a flame ionization detector and a fused-silica capillary column (Omegawax 250, size: 30 m × 0.25 mm × 0.25 µm, cat. no. 24136-SUPELCO) was used. Gas flows were 1.2 mL min^{-1} for the carrier gas (H2), 30 mL min^{-1} for the make-up gas (N_2) and 30 and 300 mL min^{-1} for H_2 and synthetic air, respectively. The initial temperature for the column flame was set at 50º C, held for 2 min, and then increased to 220º C at a rate of 4ºC min^{-1}, where it remained for another 25 min. The sample's split ratio was 1:100. Peak areas were determined by comparing retention times with standards of fatty acid methyl esters (Sigma cat. no. 189-19). The data were expressed as a percentage of the area of each fatty acid.

The experiment was conducted following a completely randomized design with four treatments and eight replicates per treatment. An appropriate covariance structure for unequally spaced measures was chosen based on the fit criteria of the model. The data were submitted to the residue normality test (Shapiro-Wilk) and homogeneity of variance was determined using Levene's Test, followed by analysis of variance and polynomial regression by the PROC MIXED command of the SAS (level of significance:5%). The means were adjusted by LSMEANS and analyzed by Tukey's test adjusted by PROC MIXED.

Results and discussion

The bixin concentration of annatto was 1.8% of the dry matter. The addition of annatto as a source of carotenoid in the diet of dairy cows did not influence ($p > 0.05$) total cholesterol, HDL, LDL, and oxidized LDL i.e., LDLox (table 2). The results observed in this work may be due to the similarity of the fat content of experimental diets, since these parameters are influenced by the total fat concentration of the diet, and contrary to the observations by Ferreira et al. (2013), bixin did not promote a hypolipidemic effect, and this behavior may be justified by the carotenoid levels used in the diets.

Table 2. Total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), and oxidized LDL (LDLox) in mg dL^{-1} of the plasma of Holstein cows receiving diets containing annatto.

Variable	Experimental Diets				SEM	P - value	
	C0	C4	C5	C7		L	Q
TC	118.3	116.9	101.3	116.3	5.684	0.714	0.540
LDL	30.8	21.4	21.0	37.8	3.892	0.594	0.143
HDL	87.3	97.5	80.3	78.4	3.557	0.211	0.447
LDL ox	0.84	0.74	0.84	0.81	0.022	0.947	0.473

C0 = no annatto; C4 = dietary inclusion of annatto at 4 g kg^{-1} DM (0.07 bixin g kg^{-1} diet); C5 = dietary inclusion of annatto at 5 g kg^{-1} DM (0.09 g bixin kg^{-1} diet) and C7 = dietary inclusion of annatto at 7 g kg^{-1} DM (0.12 g bixin kg $diet^{-1}$). SEM = standard error of the mean; L = linear effects; Q = quadratic effects.

The bixin contained in annatto did not act ($p > 0.05$) as an antioxidant for lipoproteins in this study, a fact evidenced by the non-alteration of oxidized LDL (LDLox) values that should have been reduced with the inclusion of bixin. In studies involving bixin in which an increase in HDL and a reduction in LDLox (Lima et al., 2001; Paula et al., 2009; Somacal et al., 2015) were found, the diets used were hypercholesterolemic. Failure to observe these effects in the present study may be associated with the normal fat content of the diet. The antioxidant used in the diet of cows promoted change ($p < 0.05$) in values of most plasma fatty acids (Table 3).

The concentrations of saturated fatty acids C13:0 and C15:0 in cow plasma were not influenced ($p > 0.05$) by the inclusion of annatto in the feed of these animals. Plasma fatty acids C10:0, C12:0, C14:0, C16:0, C17:0, and C18:0 had quadratic behavior ($p >0 .05$).

The behavior observed for fatty acids C14:0 and C16:0 was similar to that observed for fatty acids with similar carbon numbers (C16:1, C18:0, C18:1 cis, C18:2 trans, C18:2 cis, and C18:3, respectively), which was the expected outcome of ruminal biohydrogenation. Biohydrogenation occurs by the addition of a hydrogen ion in a double bond, resulting in the conversion of unsaturated fatty acids into their corresponding saturates (Holanda, Holanda, & Mendonça, 2012).

Table 3. Profile of plasma fatty acids from Holstein cows receiving diets containing annatto

Fatty acid	Experimental diet				SEM	P - value	
	C0	C4	C5	C7		L	Q
Saturated							
C10:0	66.15	91.31	93.78	57.85	2.872	0.99	<0.01
C11:0	17.12	19.63	27.08	27.27	0.829	<0.01	0.05
C12:0	3.53	7.44	7.33	2.51	0.400	0.81	<0.01
C13:0	1.74	1.26	1.79	1.44	0.053	0.12	0.14
C14:0	15.90	8.30	9.33	9.73	0.544	<0.01	<0.01
C15:0	4.64	5.31	4.30	4.63	0.180	0.70	0.36
C16:0	155.46	137.36	131.58	151.02	1.855	<0.01	<0.01
C17:0	6.43	7.59	8.79	6.72	0.177	<0.01	<0.01
C18:0	149.86	145.65	135.52	151.34	1.285	0.11	<0.01
C20:0	1.03	1.12	7.58	5.74	0.561	<0.01	0.38
Monounsaturated							
C14:1	4.96	4.79	4.77	2.86	0.161	<0.01	<0.01
C16:1	22.35	17.13	7.01	14.89	1.002	<0.01	<0.01
C18:1 trans	259.96	256.14	264.39	257.29	0.963	0.94	0.79
C18:1 cis	261.81	260.46	263.95	278.98	1.576	<0.01	<0.01
Polyunsaturated							
C18:2 trans	5.6	6.03	5.75	3.98	0.169	<0.01	<0.01
C18:2 cis	7.48	8.50	11.09	11.03	0.292	<0.01	0.20
C18:3	0.54	0.60	1.36	1.31	0.118	0.01	0.52

C0 = no annatto; C4 = dietary inclusion of annatto at 4 g kg^{-1} DM (0.07 bixin g kg^{-1} diet); C5 = dietary inclusion of annatto at 5 g kg^{-1} DM (0.09 g bixin kg^{-1} diet) and C7 = dietary inclusion of annatto at 7 g kg^{-1} DM (0.12 g bixin kg^{-1} diet). SEM = standard error of the mean; L = linear effects; Q = quadratic effects.

The plasma fatty acid profile of animals, besides being a factor associated with their health, may be indicative of the fatty acid profile of their products, such as meat and milk. However, it is important to emphasize that, in milk producing ruminants, lipogenesis in the mammary gland influences the fatty acid profile of milk (Shingfield, Bonnet, & Scollan, 2013).

The sum of lauric (12:0), myristic (14:0), and palmitic (16:0) saturated fatty acids was higher in the control treatment, making it the most undesirable, because these fatty acids are associated with the hypercholesterolemic effect and development of cardiovascular diseases owing to their hepatic LDL receptor activity lowering effect that increases the circulating plasma LDL fraction (Dietschy, 1998).

The plasma concentration of C11:0 and C20:0 fatty acids increased linearly ($p < 0.05$) with inclusion of annatto; the increase in the fatty acid C20:0 may be related to the proportion of linoleic acid that also increased linearly with the inclusion of annatto. According to (Santos et al., 2013), linoleic acid may undergo changes in desaturation and elongation to form other ω-6 polyunsaturated fatty acids, such as gamma linolenic acid and dihomo-gamma-linolenic acids, and the latter is metabolically converted to arachidonic acid, which serves as a substrate for a wide variety of important metabolites, especially some pro-inflammatory molecules.

Arachidonic acid (C20:0) is significant in the first months of the life of animals and humans, being a constituent of cellular structures and precursors of inflammatory mediators (Schmeits et al., 1999). Annatto positively alters the plasma concentration of this fatty acid, since the milk of animals fed with this compound may exhibit higher amounts of C20:0.

The plasma concentrations of C14:1, C16:1, and C18:1 monounsaturated fatty acids in cows fed increasing levels of bixin present in annatto had quadratic behavior ($p < 0.05$), while for C18:1 trans acid, there was no influence of diet ($p > 0.05$) on its plasma concentration.

The concentration of palmitoleic acid (C16:1) was altered by the addition of annatto, and it was reduced by 68.63% in treatment C5 when compared with the control treatment (Table 3). This behavior was similar to that observed for C16: 0, but with lower intensity of reduction. Palmitoleic acid (C16:1) can be derived from diet, or via endogenous synthesis through Δ9-desaturase activity on C16:0, which adds a double bond on carbon 9 of its chain (Bessa, Alves, & Santos-Silva, 2015). The Δ9-desaturase is responsible for the

conversion of stearic acid to oleic acid (C18: 1 cis-9), palmitic acid (C16: 0) in palmitoleic acid (C16: 1 cis-9), and vaccenic acid in conjugated linoleic acid (C18: 2 cis-9 trans-11 CLA) (Palmquist & Mattos, 2011).

There was linear increase of C18:2 cis and C18:3 fatty acids, quadratic behavior of C18:2 trans ($p < 0.05$). The inclusion of annatto in the diet of dairy cows had positive effects in at the plasma level, since its inclusion at rates of 0.09 and 0.12 g of bixin kg^{-1} of DM promoted an increase in the rumenic and linoleic fatty acid concentrations, respectively.

The rumenic fatty acid, also known as conjugated linoleic acid (CLA), has been associated with beneficial effects on health. It stimulates immune response against atherosclerosis, presents hypocholesterolemic property, and acts in the prevention of diabetes mellitus and obesity, besides having strong antioxidant properties (Fuke et al., 2012; Holanda et al., 2012).

The increased concentration of such fatty acids may be related to the antioxidant function of bixin. Ruminal bacteria act through biohydrogenation in the synthesis of saturated fatty acids from polyunsaturated fatty acids, and bixin inhibits the growth of these bacteria (Majolo, Carvalho, & Wiest, 2013; Nathan et al., 2019; Yolmeh, Habibi-Najafi, Shakouri, & Hosseini, 2015).

The sums of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), and the UFA: SFA ratio of the plasma of cows fed bixin contained in annatto had a quadratic ($p < 0.05$) behavior (Table 4).

Table 4. Sums and ratios of plasma fatty acids of Holstein cows, receiving diets containing annatto

Variable	Experimental diet				SEM	P - value	
	C0	C4	C5	C7		L	Q
SFA[1]	421.85	424.97	427.08	418.26	1.014	0.55	<0.01
UFA[2]	562.71	553.66	558.32	570.34	1.352	0.026	<0.01
MUFA[3]	549.09	538.52	540.12	554.02	1.377	0.38	<0.01
PUFA[4]	13.62	15.14	18.21	16.32	0.339	<0.01	0.01
UFA:SFA	1.33	1.30	1.31	1.36	0.006	0.11	<0.01

C0 = no annatto; C4 = dietary inclusion of annatto at 4 g kg^{-1} DM (0.07 bixin g kg^{-1} diet); C5 = dietary inclusion of annatto at 5 g kg^{-1} DM (0.09 g bixin kg^{-1} diet) and C7 = dietary inclusion of annatto at 7 g kg^{-1} DM (0.12 g bixin kg^{-1} diet). SEM = standard error of the mean; L = linear effects; Q = quadratic effects. SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. [1]Sum of C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0; [2]Sum of C14:1 + C16:1 + C18:1 trans + C18:1 cis + C18:2 trans + C18:2 cis + C18:3; [3]Sum of C14:1 + C16:1 + C18:1 trans + C18:1 cis; [4]Sum of C18:2 trans + C18:2 cis + C18:3.

It is observed that the inclusion of annatto in the diet of dairy cows promoted reduction of SFA and increase of plasma UFA and MUFA, when we compared the animals that received the diet without annatto with those that received the diet with 7 g annatto kg^{-1} DM, already for the PUFAs it was observed that animals fed 5 g annatto kg^{-1} of DM had greater sum of these fatty acids.

The increase in levels of PUFA is a positive sign, as these have been associated with the reduction in the risk of cardiovascular diseases (Amaral & Oliveira, 2016; Siriwardhana, Kalupahana, & Moustaid-Moussa, 2012).

Conclusion

Bixin present in annatto does not change the levels of total cholesterol, or low- and high-density lipoproteins in dairy cows. However, it induces changes in the plasma fatty acid profile; for instance, it increases the vaccenic, linoleic, and linolenic fatty acid content, and the sum of the polyunsaturated fatty acids.

References

Amaral, J. S., & Oliveira, M. B. P. P. (2016). Avelã: composição química e efeitos benéficos associados ao seu consumo. *Riscos e Alimentos, 11*, 17-21.

Bessa, R. J. B., Alves, S. P., & Santos-Silva, J. (2015). Constraints and potentials for the nutritional modulation of the fatty acid composition of ruminant meat. *European Journal of Lipid Science and Technology, 117*(9), 1325-1344. doi: 10.1002/ejlt.201400468

Carocho, M., Morales, P., & Ferreira, I. C. F. R. (2018). Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. *Trends in Food Science & Technology, 71*, 107-120. doi: 10.1016/j.tifs.2017.11.008

Derakhshan, Z., Ferrante, M., Tadi, M., Ansari, F., Heydari, A., Hosseini, M. S., ... Sadrabad, E. K. (2018). Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. *Food and Chemical Toxicology, 114*, 108-111. doi: 10.1016/j.fct.2018.02.023

Dietschy, J. M. (1998). Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. *The Journal of Nutrition, 128*(2), 444-448. doi: 10.1093/jn/128.2.444S

Ferreira, J. M., Sousa, D. F., Dantas, M. B., Fonseca, S. G. C., Menezes, D. B., Martins, A. M. C., & Queiroz, M. G. R. (2013). Effects of Bixa orellana L. seeds on hyperlipidemia. *Phytotherapy Research, 27*(1), 144-147. doi: 10.1002/ptr.4675

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemical, 226*(1), 497-509.

Fuke, G., Nörnberg, J., Rodrigues, I., Novack, M., Bezerra, A., & Souza, A. P. (2012). Teor de CLA em leites produzidos em diferentes regiões do Estado do Rio Grande do Sul. *Revista Brasileira de Ciência Veterinária, 19*(2), 497-509. doi: 10.4322/rbcv.2014.092

Garaffo, M. A., Vassallo-Agius, R., Nengas, Y., Lembo, E., Rando, R., Maisano, R., ... Giuffrida, D. (2001). Fatty acids profile, atherogenic (IA) and thrombogenic (IT) health lipid indices, of raw roe of blue fin tuna (*Thunnus thynnus* L.) and their salted product "Bottarga". *Food and Nutrition Sciences, 2*(7), 736-743. doi: 10.4236/fns.2011.27101

Holanda, M. A. C., Holanda, M. C. R., & Mendonça Jr, A. (2012). Suplementação dietética de lipídios na concentração de ácido linoleico conjugado na gordura do leite. *Acta Veterinaria Brasilica, 5*(3), 221-229. doi: 10.21708/avb.2011.5.3.2305

Jiang, J., & Xiong, Y. L. (2016). Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat Science, 120*, 107-117. doi: 10.1016/j.meatsci.2016.04.005

Kramer, J. K. G., Fellner, V., Dugan, M. E. R., Sauer, F. D., Mossoba, M. M., & Yurawecz, M. P. (1997). Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids, 32*(11), 1219-1228. doi: 10.1007/s11745-997-0156-3

Lemos, A. R., Júnior, R., Oliveira, N., José, A. R. S., Pereira, L. A., & Silva, M. V. D. (2011). Atividade antioxidante e correlação com fenólicos totais em genótipos de Urucum (*Bixa orellana* L.). *Revista do Instituto Adolfo Lutz, 70*(1), 62-68.

Lima, L. R. P., Oliveira, T. T. d., Nagem, T. J., Pinto, A. d. S., Stringheta, P. C., Tinoco, A. L. A., & Silva, J. F. (2001). Bixina, Norbixina e Quercetina e seus efeitos no metabolismo lipídico de coelhos. *Brazilian Journal Veterinary Ressearch Animal Science, 38*(4), 196-200. doi: 10.1590/S1413-95962001000400010

López-Vargas, J. H., Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2013). Chemical, physico-chemical, technological, antibacterial and antioxidant properties of dietary fiber powder obtained from yellow passion fruit (*Passiflora edulis* var. flavicarpa) co-products. *Food Research International, 51*(2), 756-763. doi: 10.1016/j.foodres.2013.01.055

Majolo, C., Carvalho, H. H., & Wiest, J. M. (2013). Atividade antibacteriana "*in vitro*" de diferentes acessos de urucum (*Bixa orellana* L.) e sua relação com o teor de bixina presente nas sementes. *Boletim do Centro de Pesquisa de Processamento de Alimentos, 31*(1), 115-124. doi: 10.5380/cep.v31i1.32708

Nathan, V. K., Rani, M. E., Rathinasamy, G., & Dhiraviam, K. N. (2019). Antioxidant and antimicrobial potential of natural colouring pigment derived from *Bixa orellana* L. Seed Aril. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 89*(1), 137-143. doi: 10.1007/s40011-017-0927-z

National Research Council [NRC]. (2001). *Nutrient Requirements of Dairy Cattle* (7th ed.). Washington, DC: National Academy Press.

Palmquist, D. L., & Mattos, W. R. S. (2011). Metabolismo de lipídios. In T. T. Berchielli, A. V. Pires & S. G. Oliveira (Eds.), *Nutrição de Ruminantes* (Vol. 1, p. 299-322). Jaboticabal, SP: FUNEP.

Patnaik, S., Mishra, S. R., Choudhary, G. B., Panda, S. K., & Behera, M. (2011). Phytochemical investigation and simultaneously study on anticonvulsant, antidiabetic activity of different leafy extracts of *Bixa orellana* Linn. *International Journal of Pharmaceutical & Biological Archives, 2*(5), 1497-1501.

Paula, H., Pedrosa, M. L., Rossoni Júnior, J. V., Haraguchi, F. K., Santos, R. C., & Silva, M. E. (2009). Effect of an aqueous extract of annatto (Bixa orellana) seeds on lipid profile and biochemical markers of renal and

hepatic function in hipercholesterolemic rats. *Brazilian Archives of Biology and Technology, 52*(6), 1373-1378. doi: 10.1590/S1516-89132009000600008

Portela, A. S., Neto, A. N. M., Silva, P. C. D., Simões, M. O. S., & Almeida, M. G. (2014). Estatinas x ácido lipóico na prevenção e tratamento de doenças cardiovasculares. *Journal of Basic and Applied Pharmaceutical Sciences, 35*(1), 9-15.

Santos, R. D., Gagliardi, A. C. M., Xavier, H. T., Magnoni, C. D., Cassani, R., Lottenberg, A. M. P., ... Alves, R. J. (2013). I Diretriz sobre o consumo de gorduras e saúde cardiovascular. *Arquivos Brasileiros de Cardiologia, 100*(1), 1-40. doi: 10.1590/S0066-782X2013000900001

Schmeits, B. L., Cook, J. A., VanderJagt, D. J., Magnussen, M. A., Bhatt, S. K., Bobik Jr, E. G., ... Glew, R. H. (1999). Fatty acid composition of the milk lipids of women in Nepal. *Nutrition Research, 19*(9), 1339-1348. doi: 10.1016/S0271-5317(99)00091-3

Shingfield, K. J., Bonnet, M., & Scollan, N. D. (2013). Recent developments in altering the fatty acid composition of ruminant-derived foods. A*nimal, 7*(s1), 132-162. doi: 10.1017/S1751731112001681

Sigurdson, G. T., Tang, P., & Giusti, M. M. (2017). Natural colorants: Food colorants from natural sources. *Annual Review of Food Science and Technology, 8*, 261-280. doi: 030216-025923

Siriwardhana, N., Kalupahana, N. S., & Moustaid-Moussa, N. (2012). Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid. *Advances in Food and Nutrition Research, 65*, 211-222. doi: 10.1016/B978-0-12-416003-3.00013-5

Somacal, S., Figueiredo, C. G., Quatrin, A., Ruviaro, A. R., Conte, L., Augusti, P. R., ... Veiga, M. L. (2015). The antiatherogenic effect of bixin in hypercholesterolemic rabbits is associated to the improvement of lipid profile and to its antioxidant and anti-inflammatory effects. *Molecular and Cellular Biochemistry, 403*(1-2), 243-253. doi: 10.1007/s11010-015-2354-x

Van Cuong, T., & Chin, K. B. (2016). Effects of annatto (*Bixa orellana* L.) seeds powder on physicochemical properties, antioxidant and antimicrobial activities of pork patties during refrigerated storage. *Korean Journal for Food Science of Animal Resources, 36*(4), 476. doi: 10.5851/kosfa.2016.36.4.476

Yabiku, H. Y., & Takahashi, M. Y. (1992). Determinação de bixina em sementes de urucum: estudo colaborativo. *Revista do Instituto Adolfo Lutz*, *52*, 31-36.

Yolmeh, M., Habibi-Najafi, M. B., Shakouri, S., & Hosseini, F. (2015). Comparing antibacterial and antioxidant activity of annatto dye extracted by conventional and ultrasound-assisted methods. *Zahedan Journal of Research in Medical Sciences, 17*(7). doi: 10.17795/zjrms1020

Yong, Y. K., Zakaria, Z. A., Kadir, A. A., Somchit, M. N., Lian, G. E. C., & Ahmad, Z. (2013). Chemical constituents and antihistamine activity of *Bixa orellana* leaf extract. *BMC Complementary and Alternative Medicine, 13*(1), 32. doi: 10.1186/1472-6882-13-32