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EUROPEAN NETWORK TO ADVANCE CAROTENOID RESEARCH AND APPLICATIONS IN AGRO-FOOD AND HEALTH

CAROTENOIDS: PRESENCE AND FUNCTIONS IN ANIMALS

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Carotenoid presence in animals

Carotenoids are responsible for many of the red, orange, and yellow colours in animals. They are present in meat, eggs, and in the skin of fish, in the carapace of Crustaceae (**Figure 1**), and in the subcutaneous fat, the skin, the egg yolks, the liver, the integuments, and in the feathers of birds (**Figure 2**)⁹. However, since all animals utilize carotenoids, the presence of carotenoids is not limited to these previously mentioned classes. For example, carotenoids are present in all mammals in which they have important biological functions, and are found in eyes, blood, liver and adipose tissues. However, it has to be noted, concentrations found in blood may reflect days or weeks of carotenoid consumption, whereas the levels found in tissues most likely reflect consumption over periods of months and years⁴⁴.

Animal products are sources of carotenoids in human diet, but since animals as humans are not able to synthesize them *de novo*, they have to be introduced through diet. Carotenoids found in animals are directly accumulated from diet or modified by metabolic conversions. However, studies in the last two decades show that there might be exceptions among arthropods such as hemipteran and dipteran insects and mites which can synthesize carotenoids due to the transfer of carotenoid related genes from microbial to animal genome³⁹. The first evidence was by Shukolyukov and Saakov (2001)⁴³ who showed that American cockroach (*Periplaneta Americana*) could synthesize β -carotene necessary for their vision when it is not sufficient in the

diet. However, authors were not able to determine has the cockroach its own carotenoid-synthesizing enzyme set or if symbiotic bacteria in the alimentary tract were responsible for carotenoid synthesis. Furthermore, Moran and Jarvik (2010)³⁰ have shown the presence of carotenoid biosynthetic gene cluster in the genome in the pea aphid, *Acyrtosiphon pisum*, which can display red and green colour and was transferred from a fungus to an aphid ancestor.

Carotenoid profiles found in animals usually reflect the carotenoid profile of the diet. Probably the best example is a laying hen egg. A typical maize-soybean based diet will result in the presence of lutein and zeaxanthin²¹ while supplement addition in diet will result in the presence of canthaxanthin, β -apo-carotenol or citranaxanthin in egg yolks^{42, 50}. However, animals from different orders and classes vary in preference for carotenoid accumulation. Among 76 bird and mammal species used in study by Slifka *et al.* (1999)⁴⁴, birds accumulated high concentrations of oxycarotenoids, regardless of the amount in diet, while accumulation of both carotenes and xanthophylls differentiates primates from other orders. Authors also found that *Felids* (cats) had moderate to high β -carotene concentration in serum with high accumulation level while *Canids* (dogs and wolves), *Mustelids* (otters) and *Ursids* (bears) were low accumulators. Furthermore, primates show the widest



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CAROTENOIDS: PRESENCE AND FUNCTIONS IN ANIMALS

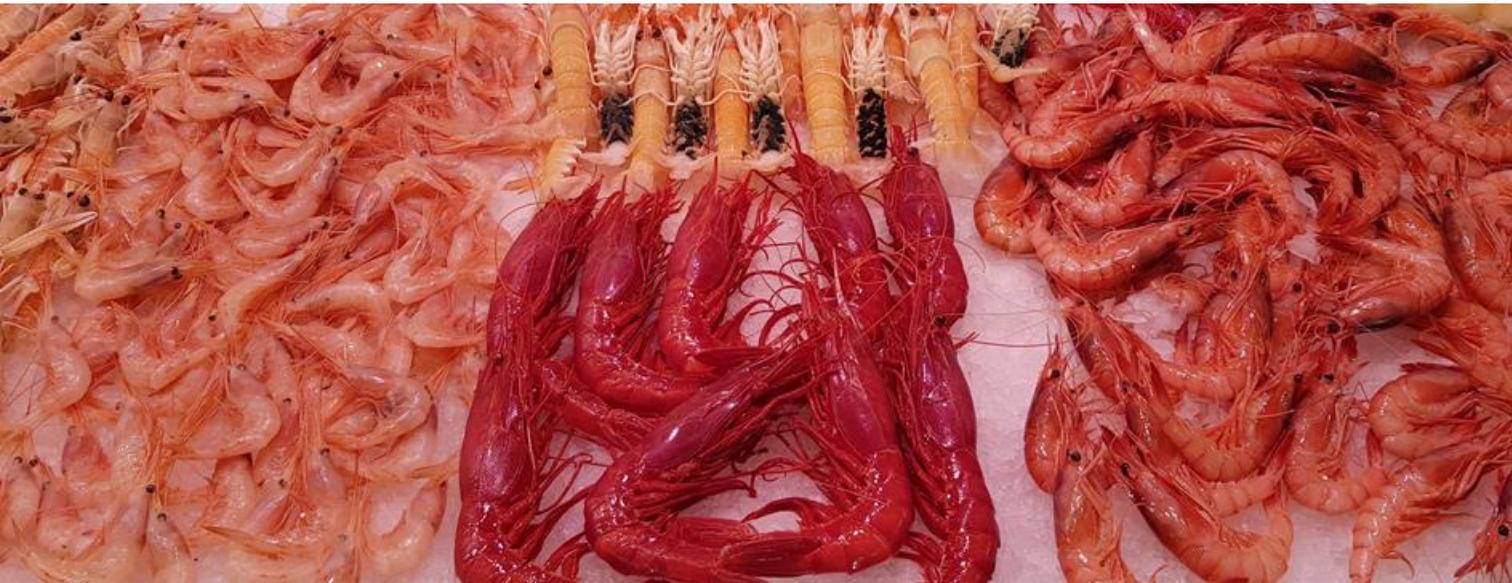


Figure 1. Colouration of the carapace of Crustaceae. Photo is reproduced with permission from K. Kljak.

array of carotenoid profiles in the blood, which include lutein, zeaxanthin, β -cryptoxanthin, lycopene, α - and β -carotene, but they are not deposited in hair like birds have in their feathers⁴⁴.

Some of the most beautiful colour combinations in nature occur in birds. Lutein, zeaxanthin, β -carotene, and β -cryptoxanthin are the major carotenoids in blood of birds, while ketocarotenoids like canthaxanthin and rhodoxanthin are responsible for colouration of feathers²³. Birds convert yellow carotenoids as lutein and zeaxanthin from diet to red ketocarotenoids as α -doradexanthin and astaxanthin via ketolation and deposit them in feathers²⁴. However, not all colouration is a result of carotenoid accumulation; parrots, for example, use psittacofulvins to colour their plumage in intensive red²⁷. Besides feathers, one of the most important carotenoid deposits in birds is in the egg yolk where they protect chick during development; this role of carotenoids will be explained in more details further in the text.

Marine animals display vivid colours as a result of accumulation of some unique carotenoids – more than 250 among 750 reported carotenoids have marine origin. Majority of them, as metabolites of diatoxanthin, diadinoxanthin and alloxanthin found in bivalves

(**Figure 3**), is a result of metabolic conversion of dietary carotenoids²⁵. On the other side, remaining ones originate from symbiotic organisms; for example, aryl carotenoids, found in sponges appear to originate from their symbiotic photosynthetic and non-photosynthetic bacteria (**Figure 4A**)²⁶ while peridinin and phyroxanthin, found in some corals originate from their symbiotic dinoflagellates (**Figure 4B**)²⁵. The first naturally occurring carotenoid sulphates are found in marine sponges from order *Verongida*, and among them, bastaxanthin C is the major carotenoid sulphate (**Figure 4C**)²⁶.

Astaxanthin is the most widely distributed carotenoid in both marine and freshwater fishes. This could be a result of oxidative metabolic conversion from zeaxanthin or originate from dietary sources²⁵. In the last two decades, some new carotenoids have been reported in fishes. Some examples are shown in **Figure 5**.

Opposite to mammals, marine animals and birds, carotenoids have been less investigated in other classes. Among insects, butterflies are the ones with the most vibrant colours. Czeuczuga (1986)¹² isolated 41 different carotenoids from 114 species; among them, zeaxanthin was the most frequent one (found in 99 species). It was followed by β -cryptoxanthin,

CAROTENOIDS: PRESENCE AND FUNCTIONS IN ANIMALS

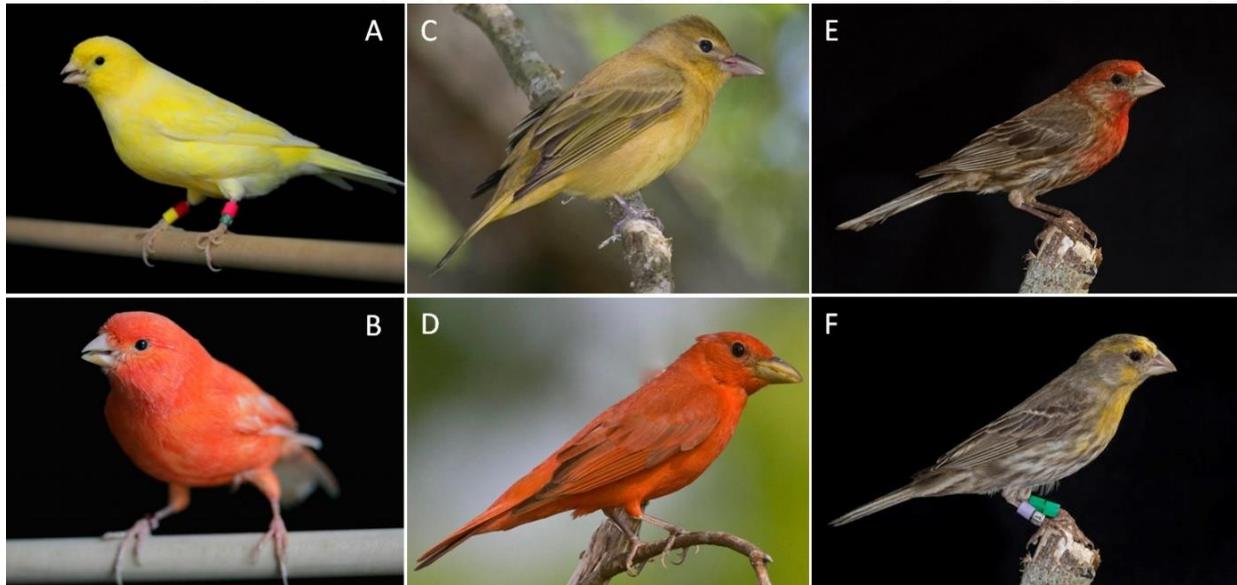


Figure 2. Colouration on feathers of birds. (A) Yellow and red (B) canaries (*Serinus canaria*), female (C) and male (D) summer tanagers (*Piranga rubra*), (E and F) male house finches (*Haemorrhous mexicanus*). Photos were reproduced with permissions from Rebecca Adrian (A and B) and Geoffrey E. Hill (C-F).

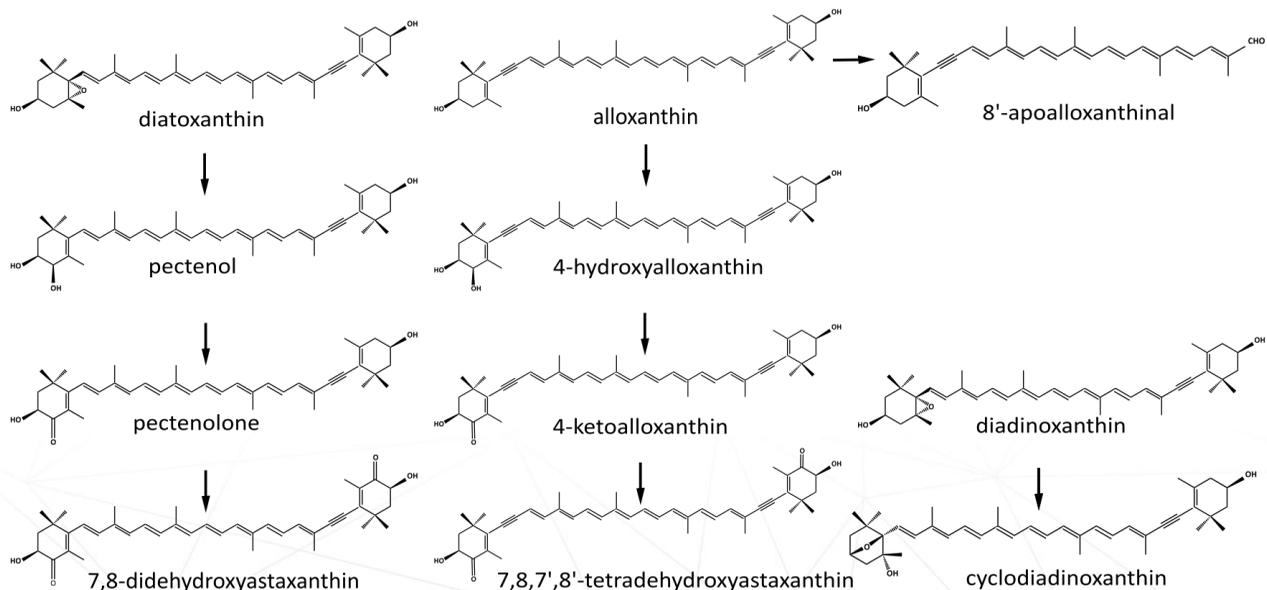


Figure 3. Diatoxanthin, alloxanthin and diadinoxanthin metabolites found in bivalves²⁵.

CAROTENOIDS: PRESENCE AND FUNCTIONS IN ANIMALS

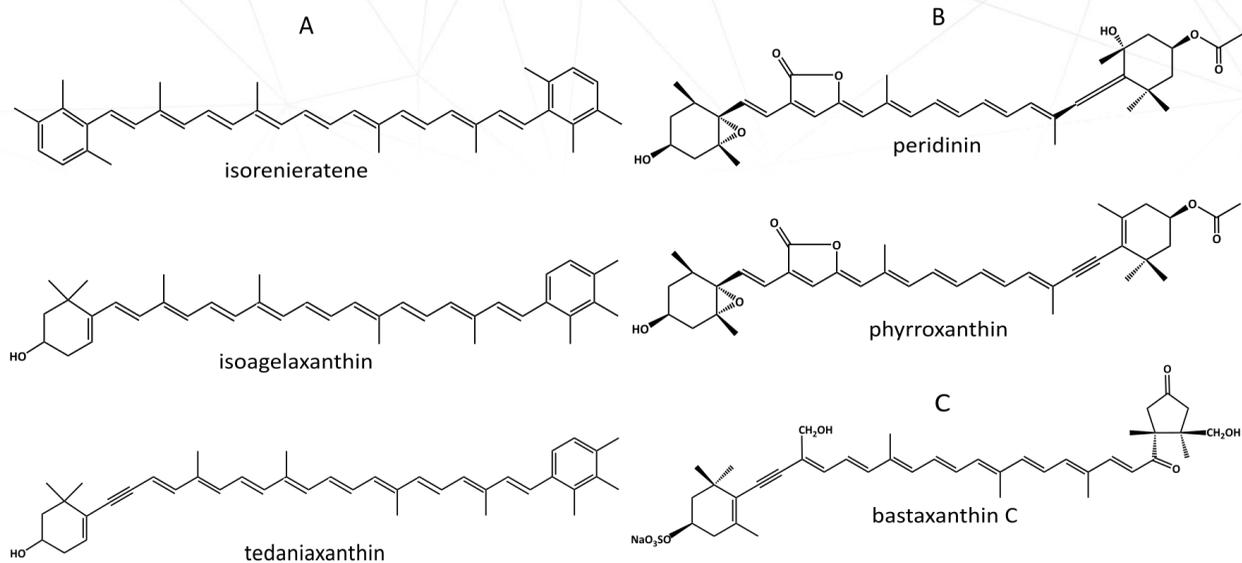


Figure 4. Aryl carotenoids isorenieratene, isoagelaxanthin and tedaniaxanthin (**A**), and bastaxanthin C (**C**) found in marine sponges. Peridinin and phyroxanthin found in corals (**B**)^{25, 26}.

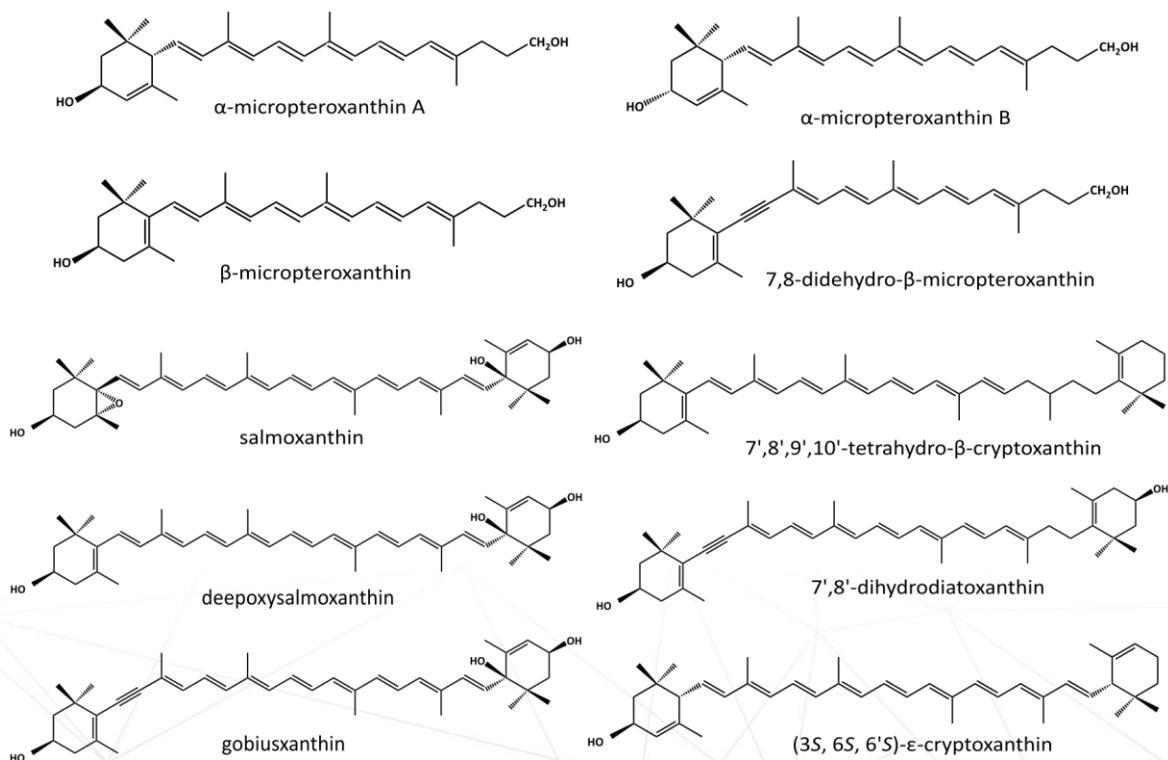


Figure 5. New carotenoids found in fishes in the last two decades²⁵.

CAROTENOIDS: PRESENCE AND FUNCTIONS IN ANIMALS

lutein epoxide, astaxanthin, lycopene, torulene (**Figure 6**) and canthaxanthin found in 84, 80, 75, 50, 47 and 42 species, respectively. Among reptiles, lizards were usually investigated. Czczuga (1980)¹¹ found that besides β -carotene, β -carotene epoxide, β -cryptoxanthin, lutein, zeaxanthin and astaxanthin, canthaxanthin, lutein epoxide, phoenicoxanthin (**Figure 6**), astaxanthin ester, mutatoxanthin (**Figure 6**) and diatoxanthin could be found in three lizard species and they are more abundant in liver and intestines than in the skin. On the other side, Raila *et al.* (2002)³⁸ have shown that green iguanas are selective accumulators of oxycarotenoids while Constantini *et al.* (2005)¹⁰ found lutein and zeaxanthin and two metabolically modified carotenoids, anhydrolutein and 3V-dehydrolutein, in the serum of Galápagos land iguanas.

Farm animals are also sources of carotenoids in human diet, and more attention is given in detection of carotenoid concentrations in their different body parts when comparing with other animal species. Additionally, the colour of animal products is the most important aspect in consumers' preference which has the highest impact in poultry and fish production. Since carotenoids have antioxidant properties, when animals are fed with appropriate amounts in diet, paler colour of their body parts or products implies that some of the carotenoids might be used in health recovery. Indeed, Ruff *et al.* (1974)⁴¹ have shown that chickens infected with coccidia endoparasites have decreased intestinal absorptive function of carotenoids and consequently lower levels of blood carotenoids than uninfected birds. In the newer study, Nogareda *et al.* (2016)³¹ have

shown that high-carotenoid diet with enriched maize had health-promoting effects in reduction of severity of coccidiosis symptoms (a delay in the parasite life cycle and reducing the oocyst load in the faeces) when compared to conventional maize diet in commercial-type broilers. Hence, the colour signifies animal's health status. For that reason, carotenoids are supplemented in animal's diet to the amount that closely reflects colour of the product originating from free-range poultry or wild fish⁵. Colour of these products could be determined using colour fans available for egg yolk, broiler skin and salmon meat colour. (**Figure 7**). Furthermore, since nowadays more attention is given to raise animals in conditions comparable to their wild counterparts', carotenoids found in tissues of domestic animals could serve as indicators of free-range or organic production^{15, 50}.

Domestic mammals are grouped into two main types: "white fat" and "yellow-fat" animals. Swine, sheep and goat hardly absorb carotenoids and they are traditionally grouped into white type while cattle and horses are members of yellow-fat domestic animals due to the accumulation of carotenoids in their adipose tissues⁴⁵. When comparing sheep, goats and steers, Yang *et al.* (1992)⁵⁷ found that steers have the highest concentration of lutein and β -carotene in serum (2.25 vs. 0.006 and 0.004 $\mu\text{g}/\text{mL}$), liver (7.32 vs. 0.87 and 0.69 $\mu\text{g}/\text{g}$) and subcutaneous fat (0.97 vs. 0.016 and 0.010 $\mu\text{g}/\text{g}$) when compared to sheep and goats, respectively. However, depending on the diet, these values vary considerably. For example, grass-fed steers had six-fold higher content of β -carotene than grain-fed steers (0.45

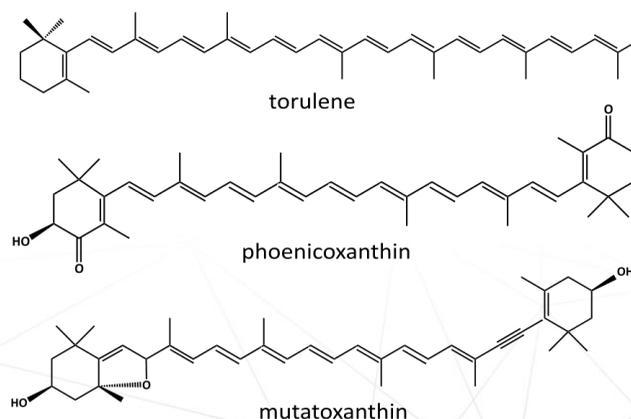


Figure 6. Torulene, phoenicoxanthin and mutatoxanthin.

CAROTENOIDS: PRESENCE AND FUNCTIONS IN ANIMALS

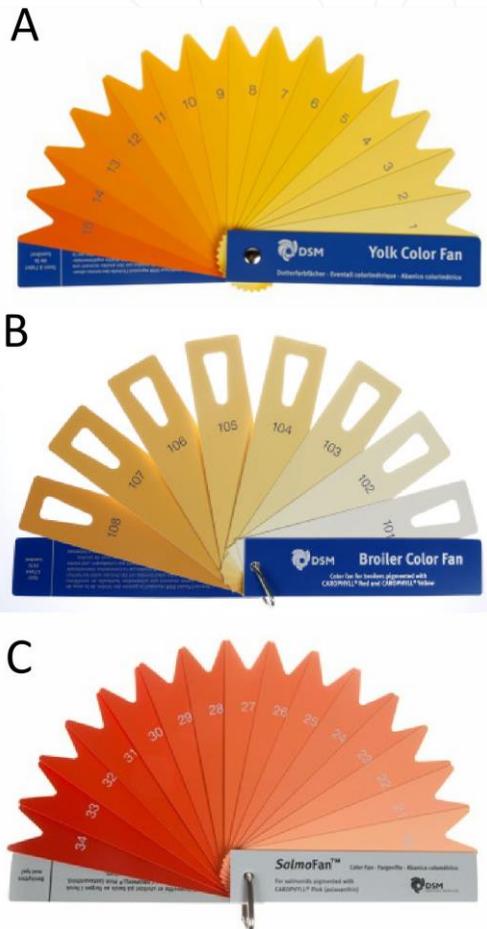


Figure 7. Colour fans for determination of colour intensity in egg yolks (A), broilers (B) and salmon (C).

vs. 0.08 µg/g tissue) due to the higher content of carotenoids in the grass¹³.

Studies of carotenoids in bovine or non-bovine milk are very sparse and concentrated on vitamin A content and β-carotene transfer to vitamin A³⁶. Milk of sheep, goats and buffaloes is whiter and mostly contain higher amounts of colourless vitamin A than cow's milk since all β-carotene is converted to vitamin A in sheep, goats and buffaloes¹⁶. Milk of these animals contains only trace amount of carotenoids³² which suggest that xanthophylls are not absorbed or not deposited in milk fat but also that they might be converted to vitamin A. Yang *et al.* (2002)⁵⁸ noticed that whiter body fat in sheep and goat could be attributed to different mechanism of absorption, metabolism and deposition of carotenoids among small ruminants and cattle.

Dairy cows transfer carotenoids to milk and two principal carotenoids are determined in milk: β-carotene is major (85%) carotenoid followed by lutein which concentration is 10-fold lower while zeaxanthin and β-cryptoxanthin could be found in trace concentrations^{32, 1}. The content of β-carotene could vary from 0.98 to 14.2 µg/g milk fat (in average 4.4 ± 0.2 compared to 0.43 ± 0.02 µg/g for lutein), with higher values in dairy cows principally grazed or fed grass silages as a main feed and with lower values in cows fed dominantly maize silages¹. Carotenoids in cow's milk are located in fat which they also colour and they are involved in sensorial properties of milk and milk products despite their low concentration. Their content in milk and plasma is influenced by cow's diet but also by cow's genotype and physiological status. In regarding genotype, Jersey cattle produce milk with higher carotenoid content than Holstein cattle⁵⁶.

In contrast to ruminant animals, diets of poultry and fish are highly influenced by consumers' preference for egg yolk, broiler and fish meat and broiler skin colour. Various pigments are available on the market, and among them capsanthin, β-apo-8'-carotenal, canthaxanthin and citranaxanthin are carotenoids not common in poultry feeds but extensively used due to the high colouring intensity. Opposite to poultry, canthaxanthin and astraxanthin are usual carotenoids in common feeds of salmon and trout⁹. However, these studies are focused on achieving desired colour of poultry and fish products, and hence, lack information on carotenoid content in animal body parts or eggs. Recently, new high-carotenoid maize hybrids were tested in poultry trials showing that additional pigment supplementation is not necessary in diets with these hybrids. Nogareda *et al.* (2016)³¹ showed high concentrations of provitamin A carotenoids in liver (3.93 µg/g of freeze-dried tissue) and zeaxanthin, β-cryptoxanthin and β-carotene in breast and thigh muscle meat (up to 5, 0.5 and 0.5 µg/g of freeze-dried tissue, respectively) in animals fed with high-carotenoid maize hybrids.

Carotenoid functions in animals

While antioxidant and provitamin A functions of carotenoids in animals are similar to carotenoid functions in humans, in this Scientific Newsletter more attention will be given to functions specific to animals. The role of carotenoids in signalling, in oil droplets for vision and in reproduction of animals will be further elaborated.

CAROTENOIDS: PRESENCE AND FUNCTIONS IN ANIMALS

Carotenoids as pigments for signalling in animals

When considering carotenoid functions in animals, pigmentation is the most prominent due to the relationship with previously mentioned food quality perception. However, this pigmentation has its purpose in animals and the most prominent is signalling. Any morphological, behavioural or chemical feature evolved because it alters behaviour of other individuals is a biological signal, and carotenoid-based signals include those for communication within and between species⁹. In that regard, signals are honest since they are costly to produce – only individuals with large carotenoid stores could use them for both body maintenance and ornamentation, or they are reliable signal of individual quality since metabolic conversion of carotenoids is linked to cellular processes and any disruption in those processes will reduce ornamentation⁵³.

The “honest” signalling is extensively researched in birds and the largest number of examples is related to their plumage ornamentation. Signalling within species includes occurrence of different colours in males and females (i.e. sexual dimorphism; as female and male summer tanagers (*Piranga rubra*) in **Figure 1**) and utilisation of colour to assess a mate for breeding⁵. The reasoning for the latter is the same as for food quality; males or females of better health status require less carotenoids acting as an antioxidant and can use more for pigmentation of sexual display⁶. In an extensive review, Møller *et al.* (2000)²⁹ showed that male fishes and birds with brighter red and yellow colour are more successful to less colourful competitors. Blount *et al.* (2000)⁷ hypothesized that feather pigmentation of female birds could indicate egg-laying ability while Pike *et al.* (2009)³⁷ have shown that three-spined stickleback (*Gasterosteus aculeatus*) female fishes prefer redder males due to their better fertility. Furthermore, occurrence of sexual display could be dependent on the breeding season; female red-bill queleas (*Quelea quelea*) loose carotenoid colouration while males retain this colouration with appearance of colouration on breast feathers varying from red to buff and on face varying from white to black⁵².

Besides sexual, another example of within-species communication is parent-offspring signalling. In a study on the rock sparrow, *Petronia petronia*, Griggio *et al.*

(2009)¹⁸ showed that nestlings with enlarged yellow, carotenoid-based breast patch ornaments were fed more frequently and defended more actively from a dummy predator than ones with smaller ornamentation. This increased parental care will likely result in enhanced survival prospects of nestlings with enlarged ornamentation.

Carotenoids contribute to between-species communication in terms of animals' warning (aposematic) signalling, mimicry, and crypsis (camouflage), but only few examples have been studied so far⁸. Warning signalling evolved through natural selection to visually display that prey is unpalatable or toxic to predators⁵⁵. For example, kite swallowtail butterflies (*Graphium* spp.) circulate lutein in their wing veins which gives yellow-green or emerald colouration to the wings⁴⁰. Regarding crypsis, there are examples where carotenoids contribute to cryptic colouration in combination with other pigments, as in stick insect (*Dixippus morosus*) with α - and β -carotene, or they are bound in carotenoid-protein complexes giving blue, green and grey colour in marine animals⁸.

Carotenoids in oil droplets of animal eyes

Primate eye contains macula, and lutein and zeaxanthin are usually found within. Their biological role includes antioxidant, anti-inflammatory and structural actions²⁰. However, other animals lack maculas, oil droplets rich in similar carotenoids are found in the eyes of birds, amphibians, reptiles, and fishes¹⁹. These oil droplets consist of colourless neutral lipids and a wide range of carotenoids that give colour from transparent to brilliant red in different cone subtypes⁴⁸.

According to the Toomey and Corbo (2017)⁴⁸, two main functions of oil droplets are acting as intracellular microlenses that enhance light delivery to the outer segment, and acting as filters of the spectrum of light reaching the outer segment. The carotenoids found in oil droplets serve as light filters, which are reducing the amount of light reaching the outer segment, and their concentrations are rarely affected by dietary carotenoid levels and immune system activation. Since spectral filtering by cone oil droplets has important role in shaping spectral sensitivity, colour discrimination, and colour constancy and, carotenoid concentration in them

CAROTENOIDS: PRESENCE AND FUNCTIONS IN ANIMALS

is relatively stable in terms of environmental and physiological effects^{48, 51}. If some small changes in oil droplet spectral filtering occur as a result of diet manipulations, they have limited impact on visually guided behaviours⁴⁸.

The cone oil droplets types vary in colour as a result of accumulation of different carotenoids and these types and carotenoids vary across species and classes. Each droplet type contains predominant carotenoid determining the long-wavelength of the spectral filtering cut-off within while other present minor carotenoids may enhance the filtering in the short-wavelength region⁴⁹. For example, in avian species, astaxanthin is the predominant carotenoid in red (R-type), zeaxanthin in green (Z-type), and galloxanthin in blue (C-type) oil droplets^{17, 49}.

Carotenoid role in the reproduction of animals

Carotenoids are found in the reproductive tissue of many mammals, including humans, and their effect on fertility of farm animals has been extensively researched for cattle, horses and pigs. In that regard, β -carotene is the carotenoid with the highest effect. High concentrations of β -carotene in bovine corpus luteum have led to questioning whether it has a specific reproduction function¹⁴. The first evidence for role of β -carotene in reproduction was that β -carotene is an integral part of microsomal membrane of bovine luteal cells³³, followed by stimulation of progesterone production by bovine luteal cells in *in vitro* conditions⁴⁶. Nowadays, dairy ruminants are more frequently housed indoor without access to green forages rich in carotenoids, especially β -carotene, which steers to required diet supplementation. As an example, Archegea *et al.* (1998)⁴ reported that timed artificial insemination in combination with β -carotene supplementation resulted in improved pregnancy rates during heat stress.

Reproduction of mammals is accompanied with the production of free radicals where β -carotene's role as an antioxidant exceeds the one of vitamin A. In more recent studies, Oliveira *et al.* (2015)³⁵ has shown that supplementation of β -carotene before calving increases blood concentration in periparturient dairy cows, while prepartum supplementation of multiparous cows with β -carotene was associated with a lower incidence of

retained placenta although it did not affect uterine health. Furthermore, β -supplemented goats showed increased ovulation rate and larger P4 (progesterone) synthesis essential for successful pregnancy³.

The evidence is growing that carotenoids have a role in the reproduction of other classes, as well. Ogilvy *et al.* (2012)³⁴ have shown that introducing lutein and β -carotene sources in the diet of red-eye tree frogs (*Agalychnis callidryas*) has a positive effect on fecundity. Similarly, astaxanthin is required for optimum reproduction in rainbow trout (*Oncorhynchus mykiss*), increasing the survival rate and lowering the mortality rate during embryonic development, and although its concentration in the male reproductive organs was low, it is increasing the fertilization rate². Furthermore, Tizkar *et al.* (2015)⁴⁷ has shown that astaxanthin dietary supplementation improves sperm quality in goldfishes (*Carassius auratus*); supplementation of 150 mg/kg improves osmolality, motility, fertilization rate, and sperm concentration.

When considering animals which lay eggs for reproduction, carotenoids are important for the survival of their offspring. For example, carotenoids which female birds deposit in egg yolk protect cells and tissues of developing chicks. Since embryos are less susceptible to free-radical attack during development when mothers deposit more dietary supplemented carotenoids into egg yolk, McGraw *et al.* (2005)²⁷ found that carotenoid-enriched eggs of zebra finches (*Taeniopygia guttata*) fed a carotenoid supplement with lutein and zeaxanthin were more likely to hatch and fledge offspring than one without supplementation. Furthermore, females fed carotenoid supplements fledged proportionally more sons than ones without pigmentation, and sons hatched from eggs containing high carotenoid levels developed sexually attractive red beaks as adults.

Concluding remarks

The high diversity and wide distribution of carotenoids in nature undoubtedly imply important biological functions. In animals they act as antioxidants and are involved in vision and reproduction while their provision of colouration for animals allows within- and between-species signalling. Due to the effect on animal production and wellbeing, carotenoid supplementation effects were evaluated in large number of studies

CAROTENOIDS: PRESENCE AND FUNCTIONS IN ANIMALS

conducted on farm animals. However, despite overwhelming evidence of the beneficial effects of carotenoids in high-producing animals, a new finding in canaries²² indicates that antioxidant aspects of carotenoid acting should be reevaluated⁵⁴. Furthermore, although it is generally accepted that animals need to obtain carotenoids through dietary intake, research in the last two decades has shown that there are exceptions among arthropods. There might be even more exceptions and future research could reveal more information about the origin of this ability.

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CAROTENOIDS: PRESENCE AND FUNCTIONS IN ANIMALS

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