

FACTORS WHICH INFLUENCE PIGMENTATION

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Introduction

Did you know that more than 90 % of the sensory perception of humans is realised by the eye?

In other species also, visual stimuli are important. This particularly applies to birds and may have to do with the fact that here other stimuli are less well developed. Contrary to mammals, smell and taste stimuli play a minor role.

Not without reason, nature thus probably saw to it that only male birds have magnificent feathers and a loudly coloured comb and wattle. The colourful plumage and shiny red comb of cocks can act as a deterrent to possible rivals when trying to win the favour of hens and, at the same time, should attract the attention of the other sex. Here colours serve for reproduction in the wider sense.

In this context, colours are of subordinate importance in humans. When choosing food, however, colours and appearance play an important role. These two parameters are relevant in judging the quality of food. Only a small number of further processed products is produced without colourants which demonstrates how important colour is for humans.

For marketing of poultry products, appearance and colour have a central role in judging quality. Consumers rate the yolk of an egg as inferior if the dose of oxycarotenoids is insufficient. In regions where maize is traditionally grown, chicks with white skin are rarely marketed and when buying young hens the yellow colour of legs and beak are a quality criterion.

In the following, the factors which influence the pigmentation of egg yolk and skin should be considered.

Oxycarotenoids are responsible for the pigmentation of egg yolk and skin as well as of legs, beak, comb and feathers and as poultry cannot produce these substances, they must be added via the feed.

Oxycarotenoid source

In nature, oxycarotenoids are found in many raw materials including various components of poultry feed. Choice of raw materials used in poultry feed is therefore a major influencing factor in pigmentation.

Maize, wheat and barley which are major feed components can cause significant variations in egg yolk pigmentation (see Table 1). The visual colour assessment was made using a Hoffmann La Roche yolk fan. The maize-containing feed led to a fan value of 10, whereas wheat and barley showed extremely pale yolks with a fan value of 4. The reason for these differences, of course, is due to the different oxycarotenoid contents in the raw materials used.

Table 1: Influence of the cereal type on different egg quality parameters

Main cereal type	Egg weight (g)	Shell weight (g)	Haugh units	HLR fan value
Maize	62.3	5.57	72.7	10.2
Wheat	61.5	5.46	75.3	4.0
Barley	62.2	5.68	76.1	3.8

according to Leeson and Summers (1997)

Oxycarotenoid content of components

Table 2 provides an overview of the most important feed components that affect pigmentation. The most important oxycarotenoid source for layer and broiler feeds is maize together with its by-products. Especially high fan values can be found in the high-protein maize by-product, which contain a high percentage of the outer part of the maize kernel. Furthermore, alfalfa and grass meal are also used. It is known from the literature and practical experience that certain sources of peas and rape may supply colourants. Because of problems relating to egg taint, rape should not be used in brown layers.

Table 2: Carotenoid content of various feed components

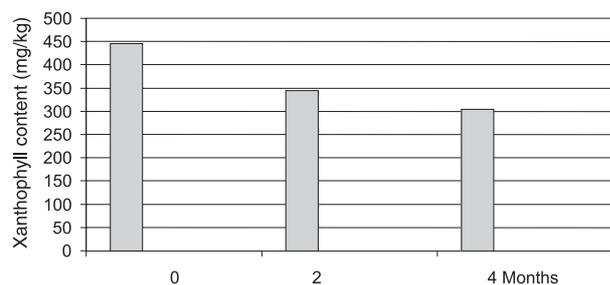
Carotenoid source	Average oxycarotenoid content (mg/kg)
Maize (US, F)	18
Maize (D)	15
Platamaize	32
Maize gluten meal (20 % XP)	20
"Maisarin" (23 % XP)	40
Maize gluten meal (50 % XP)	185
"Concentra" (60 % XP)	250
Maize gluten meal	8
Hominy feed	10
Alfalfa (17 % XP)	180
Grass meal (14 % XP)	140

Very often in practice, when optimising feed, "standard table values" are used and this is also the case for pigments. In reality, fluctuations may occur which may be due to difference in strains, different growing conditions, harvesting methods, harvesting times, storage conditions, time spent in storage, etc. When there are high levels of materials in the feed formula the real oxycarotenoid content should be analysed and, if necessary, changed in the matrix.

Influence of storage on the colourant contents

Oxycarotenoids are fat-related substances and therefore very sensitive to oxidation. This means that a certain loss of activity is unavoidable in the course of storage. Figure 1 shows this with the example of an alfalfa concentrate. Directly after drying, the xanthophyll content is approx. 440 mg/kg. After 2 months of storage the value has

Figure 1: Xanthophyll content of alfalfa (mg/kg) in the course of storage



already reduced by 23 % to 340 mg/kg. After 4 months of storage the loss of pigments amounted to 30 %. Compared to the values analysed in the fresh material, a reduction of more than 50 % may be expected for maize, maize by-products, alfalfa and grass meal when the storage period is up to 12 months. These losses in activity must be considered when formulating feed in order to avoid complaints - especially self-evident in the case of broiler skin pigmentation.

Heat treatment

Many feed additives show reduced activity due to the influence of heat during the feed production process (e.g. hot steam pelleting, expanding). Oxycarotenoids are no exception. Thus, in case of doubt, the contents should be analysed.

Supplementation of colourants

So far only raw materials have been mentioned as sources of pigments. However, egg yolk colour demanded by the consumer cannot be achieved with the usual components because this depends on the colouring effect of the oxycarotenoids included in the raw materials. All the raw materials mentioned up to now contain mainly the pure yellow-colouring lutein. Maize additionally contains the orange-colouring zeaxanthin. Even with high inclusion rates in the feed the Roche fan value cannot be raised over 10. For a more intensive yolk colour, therefore, colourants must be added.

Similar circumstances apply to skin pigmentation. The high oxycarotenoid level required in the feed necessary to reach a sufficient degree of yellowness in the epidermis cannot be achieved with the usual feed components. Yellow colourants, and in some areas, red colourants must be added.

The following table shows the usual colouring additives (Table 3). If the yellow degree of egg yolk or skin needs to be intensified, the synthetic apo-ester or standardised marigold products may alternatively be used. For the intensification of the red colour, two synthetic products - canthaxanthin and citranaxanthin - are available. Standardised paprika products may be a natural alternative.

Table 3: Standardised pigment sources of synthetic and natural origin

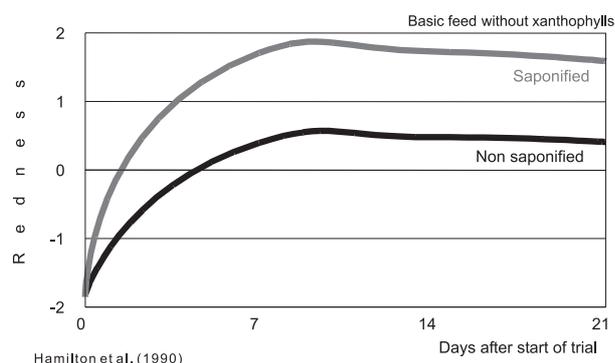
Source	Oxycarotenoid content (g/kg)
Carophyll Yellow (apo-ester)	100
Lucantin Yellow (apo-ester)	100
Different marigold products	12 - 25
Carophyll Red (canthaxanthin)	100
Lucantin Red (canthaxanthin)	100
Lucantin CX Forte (citranaxanthin)	100
Different paprika products	5 - 10

With regard to the pigment content, all colourants should be standardised with a fixed value. Because of the presence of added antioxidants, pigment activity can normally be guaranteed for one year. As the inclusion rate of pigments in feed is low, a homogeneous mixing with all the other feed components is extremely important for good colour efficiency. It is also fundamental, for marigold and paprika products, to choose the correct carrier to ensure stability and optimum mixing in the feed.

Saponification

The biological efficiency of pigments from vegetable sources depends on whether they are available in esterified or in free form. In nature, pigments in many plants are almost solely attached to fatty acids as the ester. Pigment molecule and fatty acid molecules have to be split to enable them to pass through the intestinal wall in poultry. This happens by means of saponification which more or less takes place in the intestinal tract of poultry. Many efficiency trials, however, have shown that the use of pre-saponified pigments is advantageous for pigmentation. Figure 2 illustrates this with an example of paprika.

Figure 2: The effect of saponified paprika on egg yolk pigmentation



Hamilton (1990) investigated the deposition of esterified and saponified paprika pigments by measuring the red colour of the egg yolk photometrically. Saponification seems to accelerate the deposition of pigments. This is indicated by the steeper slope of the red curve plotted for the saponified paprika product. Saponification achieves a stronger shade of red. This is due to improved resorption of the paprika products released through saponification and is confirmed by analysis of the yolk pigment content. Twice as much of the saponified paprika product was

stored as the esterified product (16 % versus 8 %). Similar results are also valid for marigold pigments. At the same inclusion level related to pigment activity, a marigold colourant attached to fatty acids certainly gives a less intensive skin pigmentation in broilers than a saponified product.

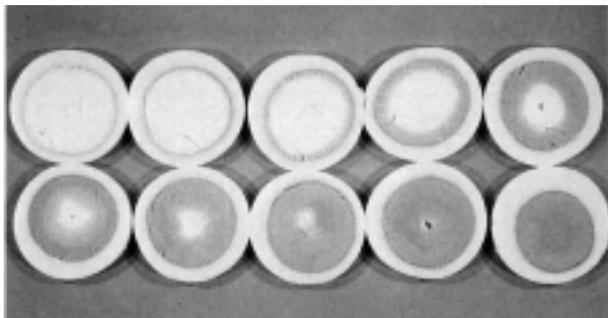
This means that, besides the absolute pigment value, the degree of saponification is also important for choosing natural pigment supplements.

Effect of incorrect feed mixtures on pigmentation

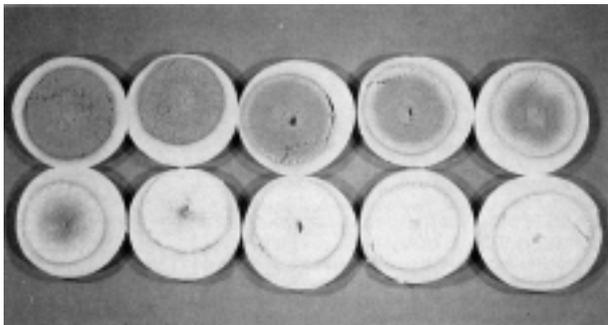
With regard to pigment supplementation, the possibility of incorrect mixtures cannot be completely excluded. Their effects can be demonstrated using the example of yolk pigmentation. Although a mature hen lays one egg almost every day, the egg, and in particular the yolk, takes 15 to 21 days to form. The yolk mass grows by having new layers laid around it. These layers can be clearly seen in hard boiled eggs in the form of so-called concentric rings. Due to this process, any change in the feed's pigment content and therefore any incorrect blend can be ascertained very quickly. Hatzipanagiotou and Hartfiel (1984) demonstrated this method in a trial with laying hens (Figure 3).

Figure 3: Egg yolk colour over the course of time - cuts of eggs laid by the same hen -

Feed supplemented with 20 mg red/kg



Feed without any addition of carotenoids



Hatzipanagiotou and Hartfiel (1984)

Note the upper part first. After a depletion period the birds were given a feed supplemented with 20 ppm of synthetic red pigment. Eggs were collected over a ten day period, the first egg at the start of the pigment supplementation of the feed. All eggs were then boiled and the colour of the yolk judged visually. A slight reddening of the yolk can clearly be seen as early as the second day after the addition of pigment, present as a narrow outer layer. From day 9 onwards, the yolk appears to have taken on a uniform colouring.

The withdrawal of colour components corresponds to the accumulation of oxycarotenoids in the yolk mass. When omitting the red pigment from the feed, the yolk appears pale on the outer layers after a few days.

As described above, it is possible to identify an incorrect blend in the case of egg yolk pigmentation within a few days. If the feed is changed immediately the loss might be limited to 6 to 8 eggs per hen. An incorrect blend cannot be recognised so quickly in the case of skin pigmentation although leg colour may be a hint for colour deviations. With regard to the skin colour of broilers, mistakes due to incorrect blends cannot usually be completely rectified during the course of the fattening period.

Feed intake

Feed intake and thus oxycarotenoid intake have an important influence on pigmentation.

The **energy content of the ration** plays a significant role, particularly in the laying hen. An increased energy content in the feed normally leads to a decreased feed intake and in such cases all relevant nutrients and active substances (including colourants) must be adjusted.

During **higher ambient temperatures** (e.g. in summer time) a decreased feed intake may also occur. Here corresponding steps - as mentioned above - can be used to counteract the situation.

A precondition for optimum feed intake is a good **feed structure**. Particularly high amounts of fine particles lead to depression of intake in laying hens. The proportion of particles under 0.5 mm should amount to less than 19 %. If the pigment supplement is among these fine particles, which the hen tends not to eat, this must lead to problems with pigmentation.

Attention must be paid to a good **pellet quality** in fattening poultry so that the animals take in enough feed.

As already mentioned in the introduction **smell and taste** play a minor role in poultry. However, under certain conditions these reactions can also be seen in poultry. e.g. with the use of bitter constituents such as medication or the use of acids. The higher the acidity, the higher is the negative influence on feed and hence water intake. A decreased feed intake can partially be seen at 0.5 to 1.0 % acid use. This particularly applies to fumaric acid and their acetates (decreasing acid-effect: fumaric acid > formic acid > acetic acid > propionic acid). Rancid fats may also lead to reduced feed intake.

As is well known, **feed and water intake** are closely related. A restricted water intake normally leads to a restricted feed intake. In the case of decreased feed intake, the watering system should always be checked (e.g. hydraulic pressure). Furthermore mycotoxins (mainly vomitoxin) as well as amino acids (tryptophan) and amino acid imbalances may influence feed intake.

Additional wheat feeding

In recent years many broiler producers use whole-wheat kernels which they provide together with supplementary broiler feed (a kind of a concentrate) in the later part of the fattening period. A basic precondition for successful "wheat feeding" is homogenous mixing with the concentrate feed. Furthermore, it is important that no separation occurs in the feeding system on the farm.

To achieve optimum skin pigmentation, it must be ensured

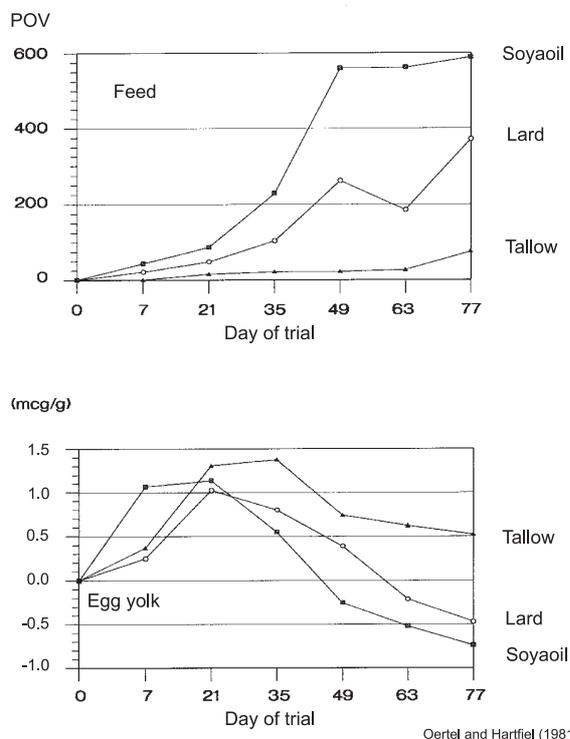
that the pigment quantity taken in with the feed is the same as that from complete feeding. The wheat, therefore, should only be administered together with supplementary feed. Colourants, as well as other nutrients and active substances (e.g. coccidiostats), must be added at the correct level based on the quantity of supplemental wheat. But also in case of an optimum composition of the supplementary feed non-uniform results in skin pigmentation - but also in live weight and with regard to fat deposition in the carcass - may occur. The reason: Chickens are able to eat selectively and in particular they prefer wheat grains. If they have the possibility to choose they will eat whole wheat rather than pelleted supplementary feed. This may cause problems, particularly if there is not enough trough area available, because the first birds will increasingly take in wheat leaving the leftovers for the following chicks.

Fat and fat quality

The resorption of fat-soluble oxycarotenoids is influenced by the fat included in the feed. Soybean oil and lard increase the oxycarotenoid deposition in the egg linearly up to a dose of 5 %. Using for example 6 % soybean oil in the feed, the citranaxanthin dosage can be decreased from 6 ppm to 4 ppm compared with the control without oil supplementation, without any change in the egg yolk pigmentation. The use of long chain, polyunsaturated fatty acids has a positive effect on the oxycarotenoid deposition, however, the use of long chain saturated fatty acids should be avoided. The contradictory results found in the literature with the use of long-chain polyunsaturated fatty acids have to do with the fact that these fatty acids have a considerably higher tendency to oxidation. In the intestinal tract oxidised fatty acids react with the oxycarotenoids and destroy them resulting in less colourants being accumulated in the yolk and skin.

The relation between fat quality and deposition of colourants was demonstrated by Oertel and Hartfiel (1981; Figure 4). The peroxide value in the feed increased over a 77-day storage period especially with soybean oil and lard, but less so with tallow. The canthaxanthin content of the egg yolk took a contrary course - shown here as a change in comparison to the initial value. In the first two weeks soybean oil causes a rapid and considerable colourant increase in the egg yolk. Afterwards the pigment content in the egg yolk decreases continuously - particularly with use of soybean oil. Thus the fat quality in poultry feed should be regarded as especially important. Oxidised fats may considerably reduce the deposition of colourants and as both fat oxidation and temperature have a negative effect it is sensible to exercise caution during the summer months.

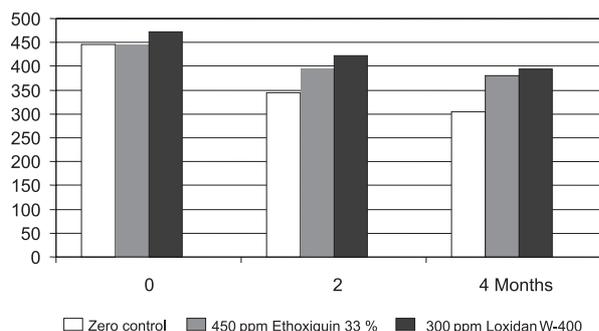
Figure 4: Influence of fat source on fat oxidation in the feed resp. on canthaxanthin content of the egg yolk



Antioxidants

Because of fat oxidation, antioxidants must be taken into consideration. Oxidation processes can be retarded or stopped using antioxidants and hence fat quality maintained for a longer time. Figure 5 shows the results of a stability test with the aforementioned alfalfa concentrate. A non-treated control was investigated with two further treatments, ethoxyquin and Loxidan (an antioxidant mixture) supplemented directly during the production. In contrast to the negative control, with a pigment loss of 30 % within 4 months of storage, the decrease with the ethoxyquin stabilisation was 14 %, and in case of Loxidan treatment only 10 %.

Figure 5: Influence of antioxidants on the xanthophyll content of alfalfa (mg/kg)



A trial by Harms, who administered ethoxyquin to laying hens via the drinking water, shows that ethoxyquin develops its protective effect mainly in the intestinal tract (Table 4). Feed intake was unchanged by treatments and yolk colour spectrum - judged by the wave length - was identical. Ethoxyquin given via the drinking water led to a significantly more intense yolk colour compared to untreated water. Synthetically produced antioxidants react with the decomposition products of the fat oxidation in the intestine and thus also protect other feed additives which are endangered by fat oxidation.

Table 4: Influence of ethoxyquin in the drinking water on egg yolk pigmentation

Ethoxyquin in water (mg/ml)	Excitation purity	Wavelength (nm)	Feed consumption (g/hen/day)
0	84.38 ^a	578.3 ^a	98.4 ^a
2.2	86.17 ^b	578.4 ^a	99.5 ^a

Besides oxycarotenoids antioxidants can also protect vitamin E against oxidation. Because of the acetate form vitamin E feed supplements are protected against oxidation during feed storage. However, in the intestinal tract vitamin E is split into free tocopherol and acetate. If oxidised fats are present in the intestinal tract any free tocopherol can act as an antioxidant and can be used up. As a result, less tocopherol is absorbed through the intestinal wall and its important function, as a biological antioxidant in the organism, is reduced. In extreme cases vitamin E deficiency symptoms may occur. In addition the negative effects regarding stability of poultry and poultry products during storage must be considered.

Vitamin E as a natural antioxidant

As already mentioned with regard to fat quality, tocopherols also act as antioxidants and their supplementation in the feed therefore has a positive effect on pigmentation. Tocopherols may protect the oxycarotenoids in the intestinal tract against oxidation but with the result that less tocopherols are absorbed as a biological antioxidant.

Calcium

The calcium content in feed is repeatedly mentioned in the literature in connection with egg yolk pigmentation. High calcium levels negatively influence yolk colour. If the calcium content is raised from 2.5 % to 3.5 % in layer feed it is necessary to include 1.7 ppm citraxanthin instead of 1.0 ppm to achieve the same yolk pigmentation. In a further trial an increase of the calcium content from 3 % to 4 % led to a decreased yolk colour of one on the Roche fan scale. There are also references in the literature to a reduction of feed intake with an increased calcium content in the ration which will result in a reduced oxycarotenoid intake. Thus the calcium content in the feed should not be adjusted higher than is necessary.

Vitamin A

We return to the vitamins. In high doses, vitamin A disturbs the absorption of oxycarotenoids because both compete for the same transport mechanism. Several

* pooled sample of 18 livers per treatment
(9 replicates each with 10 male broilers per treatment, trial period 35 days)

years ago the feed industry used this fact to include high amounts of vitamin A (up to 100,000 IU/kg) in broiler feed. A white carcass could be produced even though high amounts of maize were used - at that time maize was very cheap. Legislation was introduced to limit vitamin A during the fattening because of the possible risk of excessive vitamin A contents in foodstuffs of animal origin (e.g. liver, liver products).

Incorrect mixtures with high vitamin A contents - or also massive doses of vitamins administered via the drinking water - can be a reason for insufficient pigmentation of skin and yolk. A 3-fold overdose - 36,000 IU instead of 12,000 IU vitamin A/kg feed - leads to a decreased colourant concentration in the tissue of toes and blood plasma of almost 50 %. The content of colourant in the liver decreases by about 30 %, accompanied by a significant increase of the vitamin A concentration.

Table 5: Effect of vitamin A in broiler feed on skin pigmentation

Parameter	12,000 IU vitamin A/kg feed	36,000 IU vitamin A/kg feed
Apo-ester Concentration in:		
Toe web ($\mu\text{g}/\text{cm}^2$)	1.20	0.68
Blood plasma ($\mu\text{g}/\text{ml}$)	9.8	5.4
Liver, fresh weight ($\mu\text{g}/\text{g}$)	16.7	12.1
Vitamin A content		
Liver, fresh weight (IU/g)*	552	1.490

Growth promoters

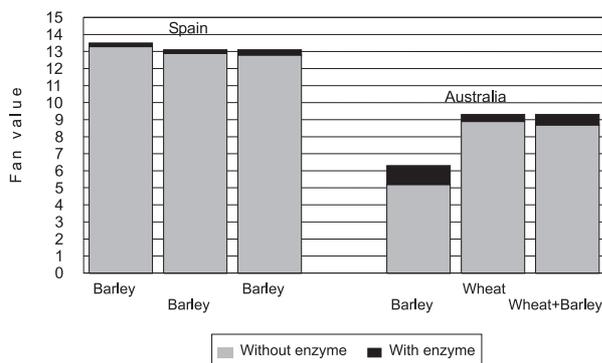
There are different findings concerning the influence of growth promoters on pigmentation. Positive effects can be seen in some trials, whereas other trials show no difference. Some authors assume, that performance promoters may improve the absorption of pigments by exerting a positive effect on intestinal health especially in situations of subclinical diseases of the intestine.

Feed ingredients with an unfavourable effect on pigmentation

It is known that barley, as a component in poultry feed, has a negative effect on pigmentation. This is explained by the fact that barley contains **non-starch-polysaccharides** (beta-glucan), which cause a higher viscosity of the intestinal content and therefore unfavourably influence the digestion and resorption of pigments. Similarly, wheat with a high pentosan content may lead to similar effects.

Supplementation with enzymes (beta-glucanase, xylanase etc.) leads to a positive effect on yolk pigmentation (Figure 6). In a trial with 68 % barley, carried out in Spain, an improvement of 0.2 according to the Roche scale was registered with very high initial fan values (13). With a significantly lower initial fan value (6 - 9), the effect of enzyme supplementation was much more evident in the Australian trial and which also applied in the wheat ration. Australian wheat is characterised by its high amounts of soluble pentosans.

Figure 6: Effect of enzymes on egg yolk pigmentation



Mycotoxins can also unfavourably influence the pigmentation of yolk and carcass. Ochratoxin in particular, as well as aflatoxin and fusarium-toxin must be mentioned in this connection. The oxycarotenoid metabolism can be changed by the presence of mycotoxins as follows:

- Dilution of oxycarotenoids in the intestine
- Reduced resorption via the intestinal wall
- Reduced transport in the serum
- Changed storage in the liver
- Changed deposition in the tissues.

As the aforementioned mycotoxins hamper fat transport in general, an indirect influence on the fat soluble oxycarotenoids can be expected. Ochratoxin and aflatoxin may be a cause for increased meat and blood spots in eggs as well as bleeding in the carcass. Aflatoxin, and also T2-toxin (source is maize), may cause intestinal resorption irritations. Fat metabolism is especially affected by a decreased lipase and bile production. Fat is excreted via the faeces.

Another feed ingredient with a negative effect on yolk pigmentation is gossypol from cottonseed. Gossypol forms complexes with iron, which lead to undesirable greenish to dark spots in the yolk.

Health

Some poultry diseases have a specially negative effect on pigmentation of yolk and carcass. Coccidiosis outbreaks occur more frequently in alternative housing systems affecting broilers but also laying hens. Even a light coccidiosis without obvious symptoms may cause reduced skin pigmentation. The coccidia type, as well as the gravity of the disease and the respective intestinal area affected, are decisive factors effecting colour. Coccidia, which settle in the anterior intestine have a stronger effect on pigmentation than, for example, caecal coccidia.

The effect of an infection of the small intestine with *Eimeria acervulina* is demonstrated in the following Table (6). Already, 3 days after the infection, the contents of lutein and canthaxanthin in the serum and in the liver have dropped dramatically. Five days after the infection canthaxanthin is hardly detectable in serum and liver and in the toe web only 30 % of the initial values were found. These figures illustrate clearly the effect of coccidiosis on pigmentation.

Table 6: Influence of a coccidia infection (*Eimeria acervulina*) on the carotenoid metabolism of broilers

Treatment	Not infected	3 d Post-infection	4 d Post-infection	5 d Post-infection
Content in serum (µg/ml)				
Lutein	4.12 ^a	2.57 ^b	2.73 ^b	1.73 ^c
Canthaxanthin	2.38 ^a	2.20 ^a	0.21 ^b	0.02 ^b
Content in the liver (µg/g fresh weight)				
Lutein	6.03 ^a	3.85 ^b	3.70 ^b	2.33 ^c
Canthaxanthin	14.81 ^a	6.51 ^b	0.20 ^c	0.31 ^c
Content in the toe web (ng/cm ²)				
Lutein	118 ^a	106 ^a	112 ^a	102 ^a
Canthaxanthin	31 ^a	19 ^b	6 ^c	10 ^c

according to Tyczkowski et al. (1991)

animals: male broilers

infection on 15th day of life with *Eimeria acervulina* (1,500,000 spores/animal);

at indicated times 4 birds per treatment were killed to obtain serum, liver and toe web samples for xanthophyll determination

There are several other diseases that show a direct influence on pigmentation. Diseases of the intestine, Contagious Avian Coryza, Newcastle Disease as well as helminthosis belong to this category. Similarly, Fatty Liver Syndrome in laying hens may lead to alterations in pigmentation. Thus, it can be said that a consistent colouring of carcass and egg yolk may be regarded as an indicator of good health and good practical hygienic conditions.

Coccidiostats and helminthica

It is known that the use of nicarbazin or piperacin causes spots in egg yolks.

Influence of the housing system

The influence of different housing systems, which is often mentioned in the literature, mainly depends on differences in feed intake and hygienic status. A trial carried out in the USA (Allen, 1993) shows, how the hygienic status may influence pigmentation. An additional fumigation of the broiler house with formaldehyde resulted in higher lutein contents in the blood plasma and in the toe web of 3 week-old broilers at both pigment doses than when classical wet cleaning was used. Also, body weight was significantly increased with a formaldehyde treatment (see Table 7).

Table 7: Influence of the hygiene level of housing on carcass pigmentation in broilers

Treatment	Total xanthophylls per feed (mg/kg)	Average of 3 strains
Lutein content in the blood serum (µg/ml)		
Standard	8.94	3.14
Formaldehyde	8.94	4.29
Standard	41.4	8.31
Formaldehyde	41.4	11.04
Lutein content in the toe tissue		
Standard	8.94	38.88
Formaldehyde	8.94	47.87
Standard	41.4	63.78
Formaldehyde	41.4	98.10

