VITAMIN A METABOLISM IN RECESSIVE WHITE CANARIES

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Final Acceptance: 1 July 1999

Abstract *Animal Welfare 2000, 9:* 153-165

*In recent years, a possible defect in vitamin A metabolism in recessive white canaries (*Serinus canaria*) has been repeatedly discussed. It has widely been accepted that a reduced absorption of carotenoids from the small intestine results in an insufficient synthesis of vitamin A. Moreover, the uptake of vitamin A from the lower intestine has also been discussed.*

The aim of the present study was to investigate the utilization of ß-carotene and vitamin A by recessive white canaries (in comparison to coloured ones) as well as to quantify the accretion of vitamin A in the liver and vitamin A levels in plasma and fat tissues of canaries fed different doses of ß-carotene (≈ 6000iu vitamin A kg^{-1} diet) vs vitamin A (6000 or 18 000iu kg^{-1} diet).

The results were as follows:

i) coloured canaries supplied exclusively with ß-carotene maintained normal vitamin A levels in the liver. These data indicated that conversion rates of ß-carotene to vitamin A (as established for poultry) were appropriate;

ii) recessive white canaries were totally unable to utilize ß-carotene (based on vitamin A levels in blood, liver and fat);

iii) in comparison to coloured canaries, their efficiency in utilizing retinol was significantly lower. They needed three times the vitamin A intake of coloured canaries to achieve the same vitamin A levels in the liver;

iv) plasma vitamin A levels in coloured canaries did not reflect the vitamin A supply, but this blood level could be used to determine vitamin A status in recessive white birds.

Recommendations of vitamin A supplements for recessive white canaries should be given based on these data.

Keywords: *animal welfare, ß-carotene, liver, pet birds, recessive white canaries, vitamin A*

Introduction

Vitamin A has a variety of functions as a fat-soluble vitamin. The normal structure of epithelial tissues, for example (skin, cornea of the eye and mucous membranes of the respiratory, digestive and urogenital tract), is vitamin A-dependent (Sporn *et al* 1984). Furthermore, vitamin A is a growth factor that is involved in the regulation of osteoblasts' and osteoclasts' activities (bone building and bone removing cells; Buddecke 1985; Hanck *et al* 1991).

The natural vitamin A supply of granivorous birds is only guaranteed indirectly via the intake of vegetable materials which contain only ß-carotene as a precursor of vitamin A (Isler 1971; Goodwin 1986). However, in the seeds usually consumed by these birds this provitamin amounts to less than 10mg kg^{-1}. For example, white millet has an average content of 1.8, red millet 3.4 and canary seed only 0.33mg ß-carotene kg^{-1} feed (Earle & Clarke 1991).

ß-carotene is localized in the chloroplasts of plant cells, and after being eaten it is released through carotinases and taken up by diffusion into the mucosa cells of the small intestine (Bauernfeind 1972). After absorption, ß-carotene and other provitamins A are converted by the microsomal enzymes lecithin:retinol-acyltransferase and CoA:retinol-acyltransferase into retinol (Ong *et al* 1991; Ong 1993) and esterified into retinylpalmitate and retinylstearate. After incorporation into chylomicrons, the retinylester is delivered into the lymph where it is passed on to the liver as the main storage organ (Ganguly *et al* 1959; Blomhoff *et al* 1987; Blomhoff 1994). The regulatory mechanism of ß-carotene uptake into the intestinal mucosa is still unknown. It is known that absorption and transformation of ß-carotene into vitamin A are negatively correlated with vitamin A supply to the organism (Brubacher & Weiser 1985; Skan *et al* 1989; Richter *et al* 1992; Landes 1994) and that cellular retinoid linked proteins are important for the absorption and metabolism of retinol (Kakkad & Ong 1988). In addition to the interstitial and intracellular linking proteins for retinol, retinal and retin acid, two families of retin acid nuclear receptors are known. The all-trans-retin acid receptor (RARE) always appears in three subtypes (α, ß, γ; Mangelsdorf 1994). When the RARE γ-receptor is absent, embryonic deformations consistent with a lack of vitamin A have been observed (Kastner *et al* 1995; Schweigert 1998).

In recent years, a possible genetic defect in the vitamin A metabolism of recessive white canaries (*Serinus canaria*) has been repeatedly discussed. These birds are very popular with bird breeders and fanciers. The first recorded recessive white canary specimens were bred in strains of coloured canaries in New Zealand and Great Britain in 1908. The effect of this mutation is a total inhibition of carotenoid colouring in the plumage and the adipose tissue, caused by a single autosomal recessive gene. This is a clear distinction from the dominant white variety, which has isolated areas of so-called 'lipochrome colouring' throughout the plumage, especially in the flight feathers and neck area (Dunker 1928). Because recessive white canaries enjoy great popularity among canary breeders (15 000 recessive white canaries are bred annually in Germany), large numbers of these birds have been exhibited in most bird shows on the European continent (10% of birds are recessive white canaries).

Until now, it has been widely accepted that a reduced absorption of carotenoids from the small intestine results in an insufficient synthesis of vitamin A. Moreover, a lower intestinal uptake of vitamin A (compared to birds without this defect) has also been discussed. Seed mixtures usually contain only marginal levels of vitamin A precursors (Bishop & Taylor 1963; Zwart 1978; Ryan 1988; Donoghue 1993; Heisler *et al* 1997), so that recessive white canaries depend on vitamin A supplementation to avoid a deficiency (Bielfeld 1991).

Therefore, in comparison to the requirement of domestic poultry (National Research Council 1984; Richter *et al* 1991), which amounts to 6000–8000 iu kg^{-1} diet, Dorrestein and Schrijver (1982) estimated that recessive white canaries need a higher vitamin A supplement. They assumed that 18 000iu vitamin A would meet the requirement of these birds. But this statement was made on the basis of a small number of experimental birds. The diets contained ß-carotene as well as vitamin A, so they could not distinguish between effects of supplying ß-carotene or vitamin A. Furthermore, the feed intake was not determined, meaning that it was not possible to calculate the vitamin A intake or to obtain information concerning the utilization of ß-carotene or vitamin A.

The aim of the present study was to investigate the utilization of ß-carotene and vitamin A by recessive white canaries (in comparison to coloured canaries) as well as to quantify the accretion of vitamin A in the liver in both groups of canaries fed different doses of ß-carotene or vitamin A.

Methods

Animals, housing and feeding

For these investigations, 32 coloured canaries and 32 recessive white canaries (age 1–3 years; body mass 18–24 g) were kept individually in cages 50x40x30 cm. After clinical examination and health screening (ie exclusion of coccidia, determination of plasma bile acid concentration; Zinke *et al* 1999) the animals were fed exclusively with a seed mixture (vitamin A content below the analytical detection limit) over a period of 140 days in order to eliminate any possibility of extra vitamin A prior to the experiment commencing. The birds were divided into four experimental groups (see Table 1) with the intention of achieving a uniform distribution of body weight in the four groups.

During the experiment, all groups received a seed mixture (60% rape seed, 15% canary seed, 15% millets, 5% oats, 5% hemp) without any vitamin A supplementation. Furthermore, all groups were offered rusk meal supplemented with minerals and vitamins (without vitamin A; see Table 1), which was moistened with distilled water.

Table 1 **Experimental design and vitamin A levels in the diets of the individual groups. There were 8 recessive white and 8 coloured canaries in each group.**

Group	Control	Group A	Group B	Group C
Daily ration (g)				
- seed mixture	4	4	4	4
- rusk meal	2	2	2	2
+ supplement [1]				
ß-carotene	---	10.8mg	---	---
vitamin A	---	---	72mg	216mg
≈ vitamin A equivalent [2]	n.d. [3]	6000	6000	18 000

[1] control group without any supplement; group A with addition of ß-carotene; groups B and C with addition of retinol-palmitate

[2] iu kg^{-1} diet (calculated)

[3] not detectable (below detection limit of 200iu kg^{-1})

Animals of all groups received 4g seed mixture and 2g rusk meal daily. In the control group, vitamin A was not supplemented, so the content of the total ration was below the analytical detection limit (200iu kg^{-1}). In group A, 10.8mg ß-carotene was added to the rusk meal, which corresponded to a converted vitamin A content of 18 000iu kg^{-1} rusk meal and 6000iu kg^{-1} total ration (rusk meal and seed mixture). For the calculation of ß-carotene conversion, the value of 1.667iu vitamin A mg^{-1} ß-carotene was applied, as specified for domestic poultry (National Research Council 1984). In groups B and C, the rusk meal contained 18 000 and 54 000 iu vitamin A kg^{-1} respectively due to the addition of retinol-palmitate (this resulted in a vitamin A content of 6000 and 18 000 iu kg^{-1} diet respectively for the whole ration).

The diets were offered daily at the same time: rusk meal was always fed 1h earlier to guarantee a complete intake. Refusals were registered quantitatively on the following day so that the daily feed consumption could be calculated (Wolf & Kamphues 1992). After an experimental period of 170 days, the birds were euthanased with a high dose of chloroform to obtain the vitamin A content of the liver, fat and plasma. The use of chloroform was essential because other methods would have compromised the results (eg an injection of substances into veins would have influenced the blood parameter, an injection into muscles or liver would affect the chemical and histological analyses).

Analyses

The determination of vitamin A (retinol) levels was done using high pressure liquid chromatography and UV detection at 325nm. The material was initially saponified in alkali (potassium hydroxide) and extracted with hexane. The saponified solution was taken up into 1ml of methanol, filtered and dissolved in 200ml petrol ether (hexane, pentane and ether). The determination of ß-carotene levels was done in the same way, but the confined residue was diluted in tetrahydrofurane.

The accretion rate of ß-carotene or retinol in the liver was calculated to get information on the utilization of ß-carotene or retinol in recessive white and coloured canaries.

Statistical analyses

Groups (recessive white and coloured canaries) were compared using ANOVA. Comparisons between different treatments for the same kind of bird were performed with the Student's *t*-test.

Results

Vitamin A intake

The rusk meal was accepted swiftly and led in all groups to an average consumption of 1.6g $bird^{-1}$ day^{-1}. On average, 1.8–2.5 g of seed was ingested $bird^{-1}$ day^{-1}. In groups supplemented with vitamin A, intake varied between 23 (group A; coloured canaries) and 66 (group C; recessive white canaries) iu vitamin A $bird^{-1}$ day^{-1}. The vitamin A content kg^{-1} consumed feed (see Table 2) was the same for recessive white and coloured canaries, although the intake in groups A and B was higher than predicted (7000iu kg^{-1} in contrast to 6000iu kg^{-1} diet). The reason for this was the higher proportion of rusk meal in the consumed diet.

The actual vitamin A intake met the NRC recommendations of 6000–8000 iu kg^{-1} (National Research Council 1984) and the recommendation of 18 000iu vitamin A kg^{-1} diet for recessive white canaries (Dorrestein & Schrijver 1982; Dorrestein & Kummerfeld 1995).

Table 2 **Mean ± SD vitamin A intake (data in iu bird^{-1} day^{-1} and iu kg^{-1} ingested diet) of recessive white and coloured canaries (n = 8 for all groups).**

	Vitamin A intake	
	iu bird^{-1} day^{-1}	iu kg^{-1} diet
Recessive white canaries		
control	not detectable	not detectable
group A	25.9 ± 5.76 [1]	6798 [1]
group B	26.3 ± 5.58	7209
group C	65.9 ± 18.9	18 005
Coloured canaries		
control	not detectable	not detectable
group A	23.0 ± 9.00 [1]	7099 [1]
group B	24.3 ± 7.74	7666
group C	62.1 ± 22.1	18 593

[1] 1mg ß-carotene ≈ 1.667iu vitamin A

Vitamin A levels in the liver

Values of liver mass varied between 0.47 and 0.59 g (absolute weight; see Table 3) and from 2.13 to 2.79 per cent of body weight (with no significant differences between the various groups).

Table 3 **Mean ± SD absolute (g) and relative (% of body weight) liver mass of recessive white and coloured canaries (n = 8 for all groups).**

	Recessive white canaries		Coloured canaries	
	absolute	relative	absolute	relative
control	0.52 ± 0.07	2.45 ± 0.28	0.51 ± 0.09	2.65 ± 0.37
group A	0.47 ± 0.14	2.13 ± 0.39	0.51 ± 0.10	2.72 ± 0.77
group B	0.59 ± 0.05	2.77 ± 0.08	0.50 ± 0.08	2.63 ± 0.63
group C	0.51 ± 0.08	2.42 ± 0.30	0.52 ± 0.10	2.79 ± 0.74

At comparable absolute and relative liver weights, clear differences were found in the vitamin A contents of the livers between the individual groups (see Table 4). No sex-dependent effect was observed.

In spite of a period of 140 days without any vitamin A supply, vitamin A reserves (2867iu vitamin A g^{-1} liver) were observed in the livers of coloured canaries (see Table 4). In general, the liver tissue of recessive white canaries fed a diet without any vitamin A supplementation (control) showed the lowest absolute vitamin A levels, with on average 1.53iu g^{-1}.

Table 4 **Mean ± SD vitamin A levels (iu g^{-1} liver) in the liver of recessive white and coloured canaries (n = 8 in each group). Significant differences within columns ($P < 0.05$) are marked by different superscripts. Differences within rows were significant ($P < 0.01$ in each case).**

	Vitamin A level (iu kg^{-1} diet)	Recessive white canaries	Coloured canaries
control	not detectable	1.53 ± 2.03 [a]	2867 ± 1408 [a]
group A	6000 (ß-carotene)	10.7 ± 4.30 [b]	6755 ± 1233 [b]
group B	6000 (retinol)	1602 ± 683 [c]	7872 ± 1122 [b]
group C	18 000 (retinol)	6810 ± 901 [d]	11 745 ± 2110 [c]

https://doi.org/10.1017/S0962728600022491 Published online by Cambridge University Press

The vitamin A levels in group B (6000iu kg^{-1} diet) corresponded to those of the control group (coloured canaries), where no supplement was added to the diet for 10 months. Due to the addition of retinol, there was a noticeable increase in vitamin A levels in the liver tissue of recessive white canaries. However, offering a diet with 6000iu vitamin A kg^{-1} led to vitamin A levels in the liver tissue of recessive white canaries (1602iu g^{-1}) which were not only lower than the corresponding values in coloured canaries with a comparable vitamin A intake (7872iu g^{-1} liver), but also those without any vitamin A supplement (2867iu g^{-1} liver). Only a vitamin A supplement of 18 000iu kg^{-1} diet resulted in 'normal' vitamin A levels (6810iu g^{-1}) in the liver tissue of recessive white canaries. These data corresponded to values measured in group B coloured canaries (6000 instead of 18 000 iu kg^{-1} diet). On the other hand, feeding a diet of 18 000iu vitamin A kg^{-1} to coloured canaries increased vitamin A levels to more than 11 000iu g^{-1} liver.

The accretion rate (calculated from the actual intake and the difference between vitamin A content of the liver in the control and the other groups; see Table 5) showed accretion rates between 84.1 (group C) and 99.5 per cent (group B) for coloured canaries. On the other hand, the recessive white canaries were unable to utilize ß-carotene (vitamin A levels in the liver were 0.16% of the concentration found in group A coloured canaries).

Table 5 **Intakes of vitamin A and vitamin A-equivalents during the whole experimental period (170 days) and the calculated accretion rate in the liver tissue.**

	Recessive white canaries		Coloured canaries	
	total intake (iu)	**accreted in the liver (%)**	**total intake (iu)**	**accreted in the liver (%)**
group A	4403 [1]	0.21	3910 [1]	99.4
group B	4471	35.8	5031	99.5
group C	11 203	60.8	10 557	84.1

[1] supplied by ß-carotene

Due to the reduced utilization of vitamin A by recessive white canaries, the surplus vitamin A – available for accretion in the liver – is lower and, therefore, so is the vitamin A level in the liver. On the other hand, the highest vitamin A intake in coloured canaries resulted in a reduced proportion being stored in the liver (down regulation of utilization in the case of oversupply).

Vitamin A levels in fat tissue

The nutritional state of the canaries allowed an investigation of the fat tissue (see Table 6). In the macroscopic investigation, different colours of fat tissue were observed. While the coloured canaries had orange-yellow fat in the abdomen, the corresponding tissue of recessive white canaries was rather white and looked glassy.

As expected, the vitamin A content of fat tissue was lower than that of liver. However, levels varied with respect to the amount and kind of vitamin A supplied, showing comparable trends to those found in the liver: a vitamin A intake of 18 000iu kg^{-1} resulted in vitamin A levels in the fat tissue of recessive white canaries that were even lower than the values found in coloured canaries deprived of vitamin A for 140 days prior to the start of the experiment.

https://doi.org/10.1017/S0962728600022491 Published online by Cambridge University Press

Table 6 **Mean ± SD vitamin A levels (iu g^{-1} fat tissue) in the fat tissue of recessive white and coloured canaries (n = 8 in each group). Significant differences within columns ($P < 0.05$) are marked by different superscripts. Differences within rows were significant ($P < 0.01$ in each case).**

	Vitamin A level (iu kg^{-1} diet)	Recessive white canaries	Coloured canaries
control	not detectable	23.3 ± 0.53^{a}	56.9 ± 1.40^{a}
group A	6000 (ß-carotene)	2.53 ± 1.57^{a}	22.3 ± 1.37^{b}
group B	6000 (retinol)	24.0 ± 4.06^{a}	74.9 ± 8.49^{c}
group C	18 000 (retinol)	55.1 ± 1.63^{b}	127.5 ± 10.4^{d}

Vitamin A levels in plasma

Plasma samples were collected only once, at the end of the experiment (in some samples the determination of vitamin A was not possible due to an insufficient amount of plasma). The blood of the coloured canaries was darker than that of the recessive white birds. Moreover, the plasma of the recessive white canaries was nearly clear, whereas that of the coloured canaries showed a slight yellow colouring.

The plasma vitamin A content (Table 7) of recessive white canaries (control group) was lower than that of coloured ones, and the addition of ß-carotene (group A) had no significant effect on the plasma vitamin A level.

Table 7 **Mean ± SD plasma vitamin A levels (µg ml^{-1} and *µmol* l^{-1}) of recessive white and coloured canaries. Significant differences within columns ($P < 0.05$) are marked by different superscripts. * denotes significant differences within rows ($P < 0.01$).**

		Recessive white canaries		Coloured canaries	
	vitamin A level (iu kg^{-1} diet)	n	plasma vitamin A	n	plasma vitamin A
* *control*	not detectable	4	0.58 ± 0.22^{a} *(2.02 ± 0.77)*	5	0.99 ± 0.24^{a} *(3.46 ± 0.84)*
* *group A*	6000 (ß-carotene)	6	0.35 ± 0.29^{b} *(1.22 ± 1.01)*	4	0.87 ± 0.20 *(3.03 ± 0.70)*
* *control and group A*		10	0.41 ± 0.30	9	0.93 ± 0.21
group B	6000 (retinol)	5	0.71 ± 0.14^{a} *(2.48 ± 0.49)*	6	0.98 ± 0.25^{a} *(3.42 ± 0.87)*
group C	18 000 (retinol)	7	0.78 ± 0.25^{a} *(2.72 ± 0.87)*	6	0.77 ± 0.17^{b} *(2.69 ± 0.59)*

Discussion

In the present investigation, the influence of ß-carotene and retinol on vitamin A metabolism was examined in recessive white and in coloured canaries.

The vitamin A intake amounted to about 7000 (groups A and B) or 18 000 iu kg^{-1} diet (group C). The intake in groups A and B was higher than calculated (7000iu kg^{-1} in contrast to 6000iu kg^{-1}) due to the higher proportion of rusk meal in the actual ingested diet. These vitamin intakes led to the following results.

Firstly, as mentioned before, there was no significant difference in the liver weight of recessive white or coloured canaries (Skan *et al* 1989). However, the values of liver weights

as a proportion of body weight (from 2.13% of body weight in recessive white group A canaries up to 2.79% of the body weight in coloured group C canaries) were lower than those reported by Rabehl (1995). However, in her investigations some of the animals had been in a poor nutritional state (Rabehl *et al* 1996). Good agreements exist between the present results and corresponding data from budgerigars (*Melopsittacus undulatus*) where the liver weight was 2.37 per cent of body weight, and also lovebirds (*Agapornis* spp.; 2.46%; Wolf *et al* 1995).

The vitamin A levels in the liver of the control group birds (without vitamin A or ß-carotene supplement) amounted to 1.53 (recessive white) and 2867 iu vitamin A g^{-1} liver tissue (coloured). Based on these data, it can be assumed that the depletion of reserves in the liver of the recessive white canaries for 140 days pre-trial was sufficient to achieve comparable starting conditions with regard to vitamin A status in these birds (Mejia 1986).

Different liver vitamin A levels were found in the different groups (depending on the diet) as well as between recessive white and coloured canaries. Firstly, the vitamin A levels in the liver of coloured canaries in groups A (ß-carotene; 6755iu g^{-1} liver) and B (retinol; 7872iu g^{-1} liver) confirmed the ß-carotene conversion rate specified for domestic poultry (1mg ß-carotene ≈ 1.667iu vitamin A; National Research Council 1984).

The lowest vitamin A levels were found in the liver of recessive white canaries fed a diet without vitamin A supplements (control group: 1.53iu vitamin A g^{-1} liver) or with addition of ß-carotene (10.7iu vitamin A g^{-1} liver). Such low vitamin A levels can lead to different effects. In large parrots, a focal metaplasia (disorder of the tissues) of the epithelium of the lower tongue saliva gland has been observed where vitamin A levels are lower than 50iu g^{-1} liver tissue (Zwart *et al* 1979). The vitamin A levels in the liver of various birds are shown in Table 8.

Table 8 **Vitamin A levels in the liver of several kinds of birds. Vitamin A level in the diet: * 13 500 and ** 18 300iu kg^{-1}.**

Species	Vitamin A level (iu g^{-1} liver)	Author(s)
parrot (Psittaciformes)	500–1200	Zwart *et al* 1979
pigeon (Columbidae)	1340	Stam 1965
coloured canary	1428–4154	Dorrenstein & Schrijver 1982
recessive white canary	524*–3751**	Dorrenstein & Schrijver 1982
budgerigar	136	Dorrenstein *et al* 1985

It is doubtful whether information about the vitamin A content in the livers of other species are comparable to the results of the present study, because corresponding data about vitamin supply in birds are incomplete (Zwart *et al* 1979) and a vitamin A deficit under normal conditions is not rare (Dorrestein *et al* 1985; Ryan 1988), but it can be concluded that the liver is the most important storage organ. If large amounts of vitamin A are ingested, however, some of it will be stored in fat tissue. In both recessive white and coloured canaries supplied with only ß-carotene, there were significantly lower vitamin A levels in fat tissue than in those canaries supplied with retinol; the values were even lower than in the control group, an effect yet to be explained.

Without the addition of ß-carotene or retinol, the plasma vitamin A content of recessive white canaries was lower than that of coloured ones. Furthermore, the levels in the plasma of the recessive white canaries in group A underlined that no vitamin A reserves were available.

However, the addition of retinol did have an effect on the vitamin A content of the plasma of recessive white canaries. In general, these canaries showed similar plasma vitamin A

levels to chickens supplemented with 6000iu vitamin A kg^{-1} diet (2.0–2.83 µmol l^{-1}; West *et al* 1992). The release of retinol from the liver to maintain a normal plasma status is influenced by the levels of circulating retinol in the blood, so the determination of vitamin A levels in the plasma of coloured canaries is only an indicator of the vitamin A status in cases of extreme deficiency or oversupply (Gerlach *et al* 1988). Because of the tight regulation of plasma vitamin A levels, this parameter is not suitable for a precise diagnosis of vitamin A status in coloured canaries. But, as shown in Table 7, the vitamin A status of recessive white canaries can be diagnosed from vitamin A levels in the plasma.

Furthermore, the investigations showed that after a lack of vitamin A, the supply of this vitamin is used at first to restore a normal plasma level, and only surplus vitamin A is accreted in the liver (the most important storage organ). But, because of the low vitamin A levels in the plasma, as well as in the liver, of group A recessive white canaries (supplementation of ß-carotene), it must be assumed that these birds are not able to absorb the necessary amounts of ß-carotene from the intestine for vitamin A synthesis. This low vitamin A level explains the relatively bright colour of the plasma.

Whether the poor utilization of vitamin A precursors in recessive white canaries is the result of an enzymatic defect in the carotenedeoxygenase, which predominantly occurs in the epithelial cells of the intestine, but also has a low level of activity in liver tissue (Goodman & Huang 1966) cannot be assessed at the moment. However, it seems that recessive white canaries depend on a higher vitamin A supply than other birds. In addition, retinol given orally is not utilized at a normal rate: on average 35.8 and 60.8 per cent was accreted in the liver by group B (6000iu vitamin A kg^{-1} diet) and group C (18 000iu vitamin A kg^{-1} diet) respectively. Vitamin A levels in this organ which were comparable to those found in coloured canaries could only be observed after a supplement of three times the amount of retinol.

In general, the results bear out the assumption that recessive white canaries are unable to utilize ß-carotene. This effect has also been observed in cats (*Felis cattus*; Schweigert 1988) but here the inability is found in all cats. In contrast to cats, the defect concerns only recessive white canaries.

These data underline that an adequate vitamin A intake for recessive white canaries cannot be achieved by offering feedstuffs rich in ß-carotene (vegetables, herbs, etc), but only by a sufficient supplement of vitamin A (retinol).

Summary and conclusion

The most important findings of this investigation are as follows:

i) coloured canaries can maintain normal vitamin A levels in the liver when they are supplied with ß-carotene. The observed vitamin A levels in the liver indicate that the conversion rate for ß-carotene to vitamin A (established for poultry) is also appropriate for canaries;

ii) recessive white canaries are totally unable to utilize ß-carotene (based on data from plasma, liver and fat tissue);

iii) their efficiency in utilizing retinol is significantly lower than coloured canaries'. Three times higher vitamin A intakes are needed to achieve normal vitamin A levels in the liver;

iv) in contrast to coloured canaries, the vitamin A status of recessive white canaries can be diagnosed by the vitamin A levels in plasma.

In general, recessive white canaries are unable to utilize ß-carotene. Therefore, only retinol can be used as an adequate supplement. But excessively high doses of retinol can lead to acute, subacute or chronic poisoning (Gazo *et al* 1974; Sporn *et al* 1984; Hamdoon & Rahman 1990). High intakes of vitamin A result in a hypervitaminosis A with extensive clinical symptoms from metabolic bone diseases to fractures or dyskeratosis of the beak (Frame *et al* 1974; Havivi & Guggenheim 1975; Guldner 1978; Hook 1987; Biesalski 1989; Strahberger 1990). However, the critical dose depends on many factors, such as bioavailability, duration of supply, nutritional state, status of vitamins A and E, and the animal's age. Initial damage to cell membranes has been observed in chickens after a vitamin A supplement of 15 000iu kg^{-1} and symptoms of hypervitaminosis A at 22 000iu kg^{-1} diet (Laitova 1981; Gropp *et al* 1991). In feeding experiments with broiler chickens, vitamin A concentrations of 30 000iu kg^{-1} diet led to reduced growth and an increased mortality rate (Mehringer 1987).

Due to the higher vitamin A requirement of recessive white canaries, the question of the practicability of vitamin A supplementation arises. Supplementation could be done by the addition of special products, including retinol, to the diet or to the drinking water. However, possible separation (Kamphues 1995) or oxidation may lead to a lower palatability and reduced water consumption (Wendler 1995; Wolf & Kamphues 1997). High moisture feedstuffs like fruits or vegetables will also reduce water intake from the bowl (Wolf & Kamphues 1995), so that the birds do not ingest the added amounts of vitamin. Therefore, the concept of special diets (possibly expensive) should be considered for recessive white canaries.

Animal welfare implications

Recessive white canaries are unable to utilize ß-carotene, so ß-carotene does not represent a sufficient vitamin A source. In addition, the utilization of retinol is insufficient. Therefore, recessive white canaries need a higher supply of retinol. But if recessive white and coloured canaries are kept together, the risk of a vitamin A oversupply (hypervitaminosis A) in coloured canaries has to be considered. Housing recessive white canaries separately is one possible solution.

In general, recessive white canaries need a higher vitamin A (retinol) supply compared to other birds. Consequently, licences to keep these birds should be given only to breeders who have the necessary experience. But the question remains as to whether it is biologically and ethically desirable to breed birds with a known genetic defect in vitamin A metabolism.

Investigations into the role of vitamin A in gene expression have shown that retinoids are potent morphogens during embryonic development (Schweigert 1998). Therefore, a deficiency is unavoidably associated with disorders of embryogenesis (limb deformities, early embryonic death, etc). Due to this, recessive white canaries could eventually be an interesting model for exploring the importance of vitamin A during embryogenesis.

References

Bauernfeind J C 1972 Carotenoid vitamin A precursors and analogs in foods and feeds. *Agriculture Food Chemistry 20:* 456-473

Bielfeld H 1991 *Wellensittiche und Kanarien – Arten und Haltung.* Buch und Zeit Verlagsgesellschaft: Cologne, Germany

Biesalski H K 1989 Vitamin A: indication and therapy. III: toxicity and teratogenity. *VitaMinSpur 4:* 55-65

Bishop C and Taylor T G 1963 Studies on the vitamin content of bird seeds. *Veterinary Record 75:* 688-691

Blomhoff R 1994 Transport and metabolism of vitamin A. *Nutrition Review 52:* S13-23

Blomhoff R, Berg T and Norum K R 1987 Absorption, transport and storage of retinol. *Chemical Society 27:* 169-177

Brubacher G B and Weiser H 1985 The vitamin A activity of ß-carotene. *International Journal of Vitamins and Nutrition Research 55:* 5-15

Buddecke E 1985 *Outline of Biochemistry.* De Gruyter: Berlin, New York

Donoghue S 1993 Feeding parrots: nuts, seeds and extruded feeds. *Veterinarian Technician 2:* 104-117

Dorrestein G M, Bitelaar M, Van Der Hage N M H and Zwart P 1985 Evaluation of bacteriological and mycological examination of psittacine birds. *Avian Diseases 29:* 951-962

Dorrestein G M and Kummerfeld N 1995 Pet birds. In: Gabrisch K and Zwart P (eds) *Krankheiten der Heimtiere.* Schlütersche: Hannover, Germany

Dorrestein G M and Schrijver J 1982 Een genetisch defect in de vitamine A huishouding van recessief witte kanaries. *Tijdschrift voor Diergeneeskunde 107:* 795-799

Dunker H 1928 Genetic of canaries. *Bibliography of Genetics 4:* 37-140

Earle K E and Clarke N R 1991 The nutrition of the budgerigar (*Melopsittacus undulatus*). *Journal of Nutrition 121:* 186-192

Frame B, Jackson C E, Reynolds W A and Humphrey J E 1974 Hypercalcemia and skeletal effects in chronic hypervitaminosis A. *Annual International Medicine Forum 80:* 44-48

Ganguly J, Krishnamurthy S and Mahadevan P 1959 The transport of carotenoids, vitamin A and cholesterol across the intestine of rats and chickens. *Biochemistry 71:* 756-762

Gazo M, Sladka O and Feldheim W 1974 Contribution to toxicity of retinol preparations in chickens. *Archiv für Geflügelkunde 38:* 61-64

Gerlach T, Biesalski H K and Baessleri K H 1988 Serum vitamin A-determination and its informative value for the vitamin A-status. *Zeitschrift für Ernährungswissenschaften 27:* 57-70

Goodman D W S and Huang H S 1966 Absorption and metabolism of ß-carotene and vitamin A. *Bulletin of the New York Academy of Medicine 42:* 408

Goodwin T W 1986 Metabolism, nutrition and function of carotenoids. *Annual Review of Nutrition 6:* 273-297

Gropp J M, Strahberger S and Mehringer U 1991 Excess vitamin A supply in quail and poultry chicks. In: Flachowsky G, Schone F and Henning A (eds) *Vitamins and Further Additives in the Case of Human Being and Animal* pp 18-23. 3rd Symposium, 26–27 September 1991 Jena, Germany

Guldner E 1978 *Investigations on influence of over-proportioned vitamin A on the skeleton of the laying hen.* Published DVM thesis, Berlin, Germany

Hamdoon A A and Rahman A S 1990 A study on hypervitaminosis A in broiler chicken. *Veterinary Medicine Journal Giza 38:* 115-128

Hanck A, Kuenzle C and Rehm W 1991 *Vitamin A.* Parey: Berlin, Germany

Havivi E and Guggenheim K 1975 Effect of hypervitaminosis A on composition of chick cartilage. *International Journal of Vitamins and Nutrition Research 45:* 317-325

Heisler K, Seekawer K J and Sallmann H P 1997 Vitamin contents in seeds, supplements and diets for pet birds. *1st International Symposium on Pet Bird Nutrition* pp 49-50. 3–4 October 1997 Hannover, Germany

Hook E B 1987 Response to Teratology Society statement about the risk of excessive doses of vitamin A. *Teratology 36:* 143

Isler O 1971 *Carotenoids.* Birkhäuser: Basel, Stuttgart

Kakkad B P and Ong D E 1988 Reduction of retinaldehyde bound to cellular retinol-binding protein (type II) by microsomes from rat small intestine. *Journal of Biological Chemistry 263:* 12916-12919

Kamphues J 1995 Futterzusatzstoffe – auch aus klinischer Sicht für den Tierarzt von Interesse. *Wiener Tierärztliche Monatsschrift 81:* 86-92

Kastner P, Mark P M and Chambon P 1995 Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? *Cell 83:* 859-869

Laitova L 1981 Optimization of level of vitamin A in the feed for pheasants kept in aviaries. *Zivocisna – Vyroba 26:* 303-312

Landes E 1994 The concentration of vitamin A in the liver of cattle and pigs. *Übersichten zur Tierernährung 22:* 281-320

Mangelsdorf D J 1994 Vitamin A receptors. *Nutrition Review 52:* S32-44

Mehringer U 1987 *A Contribution to the Hypervitaminosis A in Poultry.* Published DVM thesis, Munich, Germany

Mejia L A 1986 Vitamin A – nutrient interrelationships. In: Bauernfeind C (ed) *Vitamin A Deficiency and its Control* pp 65-95. Academic Press, Inc: Orlando, USA

National Research Council 1984 *Nutrient Requirements of Poultry, 8th edition.* National Academy Press: Washington, USA

Ong D E 1993 Retinoid metabolism during absorption. *Journal of Nutrition 123:* 351-359

Ong D E, Lucas P C, Kakkad B and Quick T C 1991 Ontogeny of two vitamin A-metabolizing enzymes and two retinol-binding proteins present in the small intestine of the rat. *Journal of Lipid Research 32:* 1521-1527

Rabehl N 1995 *Investigations on Body Composition and its Development in Cage Birds of Different Species (Canaries, Budgerigars, Lovebirds, Cockatiels, Amazons and Grey Parrots).* Published DVM thesis, Hannover, Germany

Rabehl N, Wolf P and Kamphues J 1996 The morphological and chemical body composition of cagebirds of different species. *DVG-Tagungsband der X. Tagung über Vogelkrankheiten* pp 1-9. 7–8 March 1996 Munich, Germany

Richter G, Lemser A and Schone F 1992 Vitamin A effectiveness of the ß-carotene in laying hens. *Archiv für Geflügelkunde 56:* 157-162

Richter G, Lemser A, Sitte E and Ludke C 1991 Investigations of vitamin A-requirement of chickens, youngs and laying hens. In: Flachowsky G, Schone F and Henning A (eds) *Vitamins and Further Additives in the Case of Human Being and Animal* pp 72-75. 3rd Symposium, 26–27 September 1991, Jena, Germany

Ryan T R 1988 Vitamin A and its deficiency in birds. *Animal Practice 2:* 35-37

Schweigert F J 1988 ß-carotene metabolism of cattle and the influence of the fertility. *Übersichten zur Tierernährung 16:* 223-246

Schweigert F J 1998 Vitamin A: metabolism, gene expression and embryonic development. *Übersichten zur Tierernährung 26:* 1-24

Skan D, Yosefov T and Friedman A 1989 The effects of vitamin A, ß-carotene and canthaxanthin on vitamin A metabolism and immune responses in the chick. *International Journal of Vitamins and Nutrition Research 59:* 245-250

Sporn M B, Roberts A B and Goodman D S 1984 *The Retinoids, Volume 1 and 2.* Academic Press Inc: Orlando, USA

Stam J W E 1965 *Een Onderzook naar de Vitamine A Behoefte bij de Duif.* Published DVM thesis, Utrecht, The Netherlands

Strahberger S R 1990 *Studies to the Vitamin A-Over-Supply in Quails and Cockerels.* Published DVM thesis, Munich, Germany

Wendler C 1995 *Investigations on Possibilities of Mineral Supply of Canaries (*Serinus canaria*) with Commercially Available Feeds.* Published DVM thesis, Hannover, Germany

West C E, Sijtsma S R, Peters H P F, Rombout J and Van der Zijpp A J 1992 Production of chickens with marginal vitamin A deficiency. *British Journal of Nutrition 68:* 283-291

Wolf P and Kamphues J 1992 Feed and water intake in canaries – influencing factors and dependences. *Kleintierpraxis 37:* 545-552

Wolf P and Kamphues J 1995 Nutrition of parrots – questions and answers. *Annual for Parrot Owners Volume II* pp 143-162. Westarp: Magdeburg, Germany

Wolf P and Kamphues J 1997 Water intake of pet birds – basic data and influencing factors. *Übersichten zur Tierernährung 25:* 223-224

Wolf P, Rabehl N and Kamphues J 1995 Investigations on body composition of different species of adult pet birds. *Proceedings of the Society of Nutrition and Physiology 4:* 57

Zinke A, Wolf P and Kummerfeld N 1999 Post-prandial levels and diagnostic value of plasma bile acid concentrations in parrots. *Kleintierpraxis 44:* 181-196

Zwart P 1978 Vitamin A-lack and stomatitis in parrots. *Praktischer Tierarzt 59:* 121-125

Zwart P, Schreurs W H P and Dorrestein G M 1979 Vitamin A deficiency in parrots. In: *Illnesses of the Zoo Animals. Proceedings of the XXIst International Symposium* pp 47-52. 13–17 June 1979 Mulhouse, France