

S16-2 The abundance of essential vitamins in food chains and its impact on avian reproduction

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Abstract Birds produce fewer or less viable eggs if the micro-nutrient requirements for reproduction cannot be met. For example, embryos from failed eggs of European sparrowhawks *Accipiter nisus* often show anomalies that indicate vitamin B2 deficiency. B2 is only produced by plants and is propagated through the food chain. Here, egg B2 concentrations were compared between habitats (great tit, *Parus major*), food chain levels (great tit and sparrowhawk), and within clutches (great tit). Invertebrates were sampled to detect food chain differences between habitats. The range of B2 concentrations was narrow and three times lower in sparrowhawks than in great tits. Repeatability of vitamin concentration within great tit clutches was low. In poor habitat, there was a skew towards low vitamin levels. Because there were differences between habitats in invertebrate density and composition, the question remains whether habitat differences in vitamin concentrations occur due to differences in food abundance or its vitamin load.

Key words Vitamin B2, Avian reproduction, Food chains, Sparrowhawk, Great tit

1 Introduction

Avian reproduction requires micro-nutrients such as minerals, vitamins and amino acids. Few examples, however, show this in wild birds, for example where essential amino acids limit clutch size (Ramsay and Houston, 1998; Selman and Houston, 1996) and calcium deficiency results in poor quality egg shell (Graveland et al., 1994). Others provide indirect evidence of lowered reproductive success due to micro-nutrient deficiencies (Bolton et al., 1992; Van den Burg, 2000). Embryos from failed eggs of birds of prey often show symptoms (Romanoff and Bauernfeind, 1942; Romanoff, 1972), including those of vitamin B2 deficiency (Van den Burg, 2000).

Avian embryos have a high demand for the vitamin riboflavin, hereafter B2 (White, 1991). B2 is a precursor of two coenzymes, flavin mononucleotide and flavin adenine dinucleotide, on which many catabolic enzymes depend (White, 1991). The β -oxidation of fats pathway, which is the most significant metabolic pathway by which the embryo generates energy from yolk lipids, is particularly sensitive to shortage of B2. Its first step involves three enzymes that all depend on flavin coenzymes: acyl CoA dehydrogenase, electron transferring flavoprotein (ETF), and ETF dehydrogenase. Under a low and narrow range of B2 concentrations, embryonic mortality occurs (Romanoff, 1972; Squires and Naber, 1993). B2 deficiency in the adult, furthermore, will normally prevent production of any eggs at all (Fabri and Kühne, 1988; Squires and Naber, 1993; White, 1991).

Because of its important role in metabolism, B2 can-

not be invested in reproduction at the expense of adult requirements (Romanoff and Romanoff, 1949; White, 1991). Moreover, birds cannot store or produce B2 (Engbersen and De Groot, 1992; Romanoff and Romanoff, 1949). As a result, B2 investment in eggs depends on daily intake (Squires and Naber, 1993). Birds of prey, such as the bird-eating European sparrowhawk (*Accipiter nisus*), may be especially vulnerable to shortages because B2 cannot accumulate in their prey. Vegetation characteristics and invertebrate fauna composition may both contribute to the B2 budget of sparrowhawks, because B2 is only produced by plants and has to be relayed through the food chain by insects and songbirds to become available to them.

Thus the aims of this study were to compare B2 concentrations among habitats via the great tit (*Parus major*), at food chain levels via the great tit and sparrowhawk, and within clutches via the great tit. Invertebrates were sampled to detect food chain differences between habitats.

2 Materials and methods

2.1 Field study procedures

This study was performed in 2002. Great tits were studied in nest boxes in three different habitats on Southwest-Veluwe, a forested area in the central Netherlands. One habitat site, "Bennekom", is in mixed forest typical of the western and southern section of Southwest-Veluwe. Another, "Ginkel A", is situated in the northern and eastern part, which is dominated by pine plantations on former moors and sand drifts. Old forest relicts persist there as well among the pine plantations, dominated by oaks (*Quercus robur*). These relicts form the third habitat site, "Ginkel B". Bennekom,

representing good tit habitat, was used as reference for the tit populations of Ginkel where, due to poorer soil (Ginkel A and B) and poorer vegetation (Ginkel A), B2 shortages were more likely to occur. Sparrowhawk nests were mostly localized in the western and southern part of Southwest-Veluwe.

Orders of egg laying were determined by daily nest visits during which eggs were marked and measured. Collection of third and last eggs from great tit nests was undertaken shortly after clutch completion. Six clutches from Bennekom were sampled entirely after their nests were abandoned upon trapping of the female (clutch size: 1×12, 3×9, 1×8, 1×7). In total, 24 sparrowhawk eggs of known position in the clutch were collected from 16 nests; and 2 eggs were collected from 8 nests. Eggs were weighed before opening and the contents were mixed. Samples were stored at -20 °C until further analysis.

In April, invertebrate density in Bennekom and Ginkel A was measured from branches about 70 cm long cut from pine and birch trees. Samples were taken from 20 trees per tree species per study site. Branches were carefully inspected and shaken above a cloth, and the invertebrates counted and identified to major groups. To compare invertebrate species composition between areas and tree species, spiders were collected as well from the lower branches of birch and pine trees by shaking. The similarity in spider faunas between every two samples was expressed as shared proportion. Cluster analysis was undertaken using average linking.

2.2 Measurement of B2

Reference samples were prepared using commercially available B2 (SIGMA) and sparrowhawk egg extract (see below). Absorption measurements were performed by a λ -25 spectrophotometer (Perkin Elmer), using extract without additional B2 as control. Between 0 and 118 $\mu\text{g/ml}$, concentration and absorption (at 456 nm) showed a straight-line relation given by the equation: concentration ($\mu\text{g/ml}$) =

absorption / 0.0142. To obtain egg extract, egg samples were mixed 1:1 with chloroform and spun for three minutes at 10000 g. Then 75 μl of the aqueous phase was used to measure the absorption spectrum between 550 and 350 nm against 75 μl B2 free albumen. Peak heights were corrected for the 550 nm absorption because of differences in protein concentration between the sample and control albumen. Samples that contained excess protein were first diluted. Correction for evaporative water loss from the eggs was estimated by multiplying the calculated B2 concentrations by egg density.

2.3 Statistics

Two standard statistical tests (Mann-Whitney, χ^2) were used, as indicated in each test result. χ^2 tests were performed in Excel. Statistical significance was accepted at $P < 0.05$.

3 Results

Within great tit clutches, there was no clear pattern of B2 allocation, and B2 concentrations were largely independent of one another (Fig. 1). B2 levels of third and last eggs from 30 nests did not differ significantly (third egg $30.3 \mu\text{g/ml} \pm 4.36$ (SD), last egg $29.0 \mu\text{g/ml} \pm 4.12$ (SD); Mann-Whitney, $P < 0.1$).

Because Ginkel A was selected as a poor food site, a low nest box occupancy could be expected; and the difference in nest box occupancy between Ginkel A and B was nearly significant (Table 1; χ_1^2 , $P = 0.05$). Clutch completion in Ginkel A and B was over 10% lower than in Bennekom, due to predation and calcium shortage. There were no significant differences between sites in clutch size or laying dates.

The variation in B2 concentrations ranged between 20 and 40 $\mu\text{g/ml}$, and was similar for all three sites (Table 2). In Bennekom, the median value for all eggs was 29.7 $\mu\text{g/ml}$. Of the poor forest habitats, only Ginkel A differed signifi-

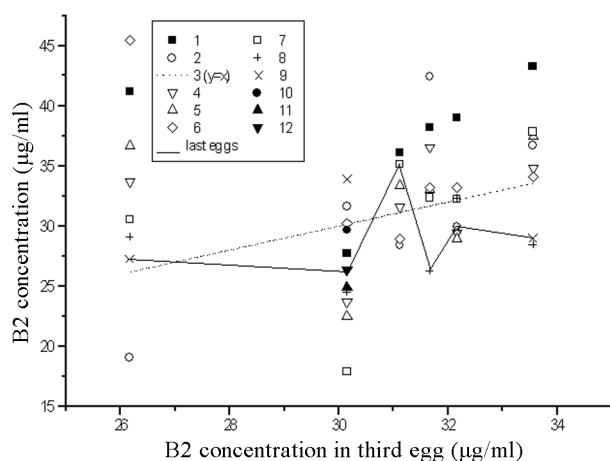


Fig. 1 Vitamin allocation in three representative great tit clutches from Bennekom

Vitamin concentrations varied irregularly and with low repeatability among eggs within clutches.

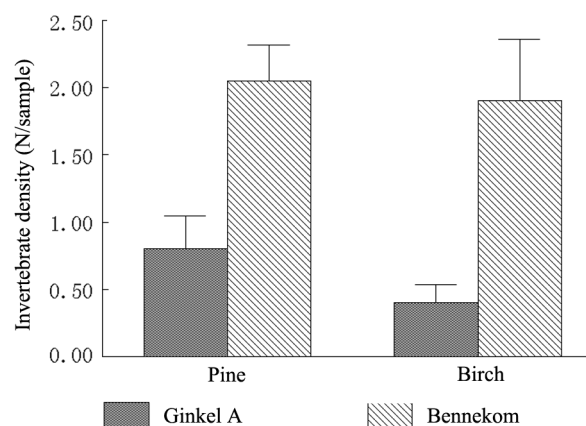


Fig. 2 Invertebrate density per study area and tree species in April

Invertebrate densities were lower in Ginkel A than in Bennekom (Mann-Whitney; $P < 0.001$).

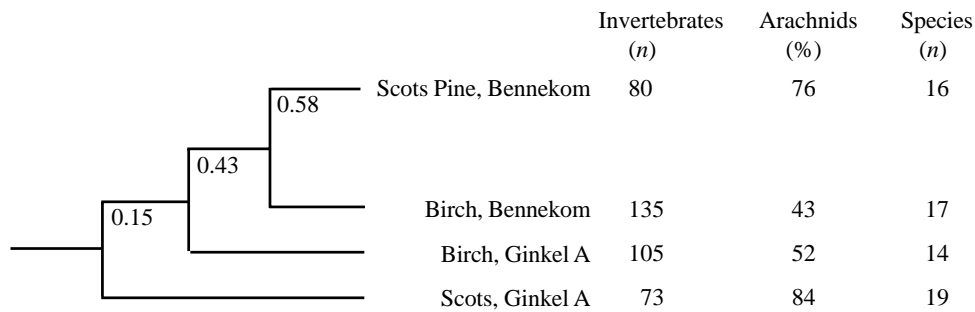


Fig. 3 Species composition of spiders per study area and tree species in April

Spiders were the dominant group among invertebrates overall. Their species composition showed specificity for both area and tree species. Similarities depict shared proportions. The comparative tree was constructed by average linkage.

cantly from Bennekom. There only 28% of the eggs exceeded the median value for Bennekom (χ_1^2 ; $P=0.035$; Table 2). Both third and last eggs showed a trend towards lower values, but it was significant for last eggs only ($n=26$ for Bennekom; $n=12$ and 11 for third and last eggs, respectively, for Ginkel A; χ_1^2 third eggs, $P=0.25$; χ_1^2 last eggs, $P=0.035$). Egg failure rates, at between 4.0% and 9.2%, did not differ between study sites ($n=93$ eggs). Five embryos were obtained from failed eggs; these did not show anomalies indicating B2 deficiency.

Prey densities on Scots pine and birch were significantly higher in Bennekom than Ginkel A (Mann-Whitney, $P<0.001$; Fig. 2). Spiders were the most common group among the invertebrates (Fig. 3). Spiders from the same tree species did not group together, indicating a site-area effect on the spider fauna. A further effect from tree species was also involved because the spider fauna on Ginkel A birch trees resembled that from Bennekom more than on adjacent Scots pines.

B2 concentrations in sparrowhawk eggs (10.84 $\mu\text{g}/$

ml, ± 1.14 (SD), range: 4.41 $\mu\text{g}/\text{ml}$, $n=24$) were about three times lower than in great tit eggs. The clutch with the lowest B2 values (8.68 and 9.25 $\mu\text{g}/\text{ml}$) was deserted during laying; the clutch containing the second lowest values (9.28 and 9.39 $\mu\text{g}/\text{ml}$) did not yield any offspring, and one of its two incubated eggs contained an embryo showing signs of B2 deficiency.

4 Discussion

Because B2 cannot be stored in the vertebrate body (Engbersen and De Groot, 1992; Romanoff and Romanoff, 1949) and not invested in eggs at the cost of female requirements (Romanoff and Romanoff, 1949; White, 1991), females can hardly make strategic decisions about B2 investment in eggs. Typically, B2 levels in eggs reflect daily intake in poultry (White, 1991). Complete saturation of riboflavin-carrier protein can be limiting; but this would result in high constant B2 levels within clutches (White, 1991), which is contrary to results from great tit nests. Therefore, it seems reasonable to assume that intra-clutch variation in B2 levels is

Table 1 Nest box occupancy (first clutches) and the proportion of clutches that were completed

Study area	Nestboxes (n)	%	Occupied (n)	Completed clutches %
Bennekom	58		55 (32)	88
Ginkel A	105		19 (20)	70
Ginkel B	87		31 (27)	74

Ginkel A had lower nest box occupancy than Ginkel B (χ_1^2 ; $P=0.05$). Nest box occupancy was highest in Bennekom, but there the nest boxes were further apart.

Table 2 Vitamin concentrations in great tit eggs from nests in which third and last eggs were analyzed

Study area	n	Median B2 concentration ($\mu\text{g}/\text{ml}$)	25% quartile ($\mu\text{g}/\text{ml}$)	75% quartile ($\mu\text{g}/\text{ml}$)	Below median	Above median	% above median
Bennekom	28	29.7	27.2	33.0	12	12	50.0
Ginkel A	18	27.4	26.6	30.8	13	5	27.8
Ginkel B	18	30.5	25.9	33.2	8	10	55.6

In Ginkel A, the distribution of vitamin concentration was skewed to the lower end of the range and few eggs contained more vitamin than the median of all eggs from Bennekom (χ_1^2 ; $P=0.035$). The skewed distribution for Ginkel A suggests a threshold for egg production between 20 and 25 $\mu\text{g}/\text{ml}$.

determined by variation in daily intake. If so, the B2 concentrations at the high end of the range are around the maximum achievable. And because these are little different from the apparent minimal levels for egg production, there may be a narrow window for egg production indeed, especially in sparrowhawks. Regrettably, birds that were unable to lay due to nutritional deficiencies remained unobserved in this study.

Romanoff and Romanoff (1949) reported substantial individual variation in B2 transfer efficiency in poultry. In the field, temporal and spatial variation in B2 availability, possibly enhanced by nonrandom habitat choice, may well obscure such differences between individuals.

Low B2 levels in great tit eggs coincided with poor habitat, in terms of soil and vegetation, low invertebrate numbers, and low nest box occupation. Because Ginkel A and B differ in B2 investments and vegetation, but not soil substrate, vegetation is the influencing factor. Differences in insect densities and species composition between the different vegetations could be correlated as well. The effect of invertebrate species composition is larger if differences in B2 load exist between species which is not unlikely.

At the other end of the food chain, sparrowhawks were expected to have difficulties obtaining sufficient amounts of B2, as their prey have no storage capacity for this vitamin. Indeed, B2 concentrations were lower and within a narrower range in sparrowhawk than great tit eggs. Because B2 uptake by the embryo depends first of all on the rate of diffusion, and thus concentration, of B2 in the yolk, B2 concentration is the biologically relevant measure of B2 availability to the embryo, notwithstanding that a sparrowhawk egg may, because of its size, hold more B2 than a great tit egg.

As B2 investments in eggs depend on habitat characteristics, B2 availability may be enhanced by habitat, especially type of vegetation and management. The question remains whether, in this case, B2 investments in the great tits of Ginkel A could be improved by an increase in food abundance as well as an increase of B2 load in their

prey.

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