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The effect of dietary amino acid composition on egg production in blue tits

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Most studies on the interaction between food supply and reproduction in animals have assumed that energy is likely to be the factor limiting egg number and/or size. In this paper, we investigate whether dietary protein proximately constrains egg production in birds. We provisioned breeding blue tits with two food supplements that differed only in the concentration of five essential amino acids. Birds receiving a supplementary diet containing an amino acid balance close to that required for egg protein formation laid significantly larger clutches (18% greater) than control birds, whereas birds receiving an otherwise identical supplementary diet but without a favourable amino acid balance did not increase egg production. To our knowledge, this is the first demonstration that dietary amino acid composition may limit egg production in free-living birds.

Keywords: tits; protein; amino acids; egg production

1. INTRODUCTION

Birds have provided convenient models for many of the classic studies on the factors influencing the level of investment made in reproduction. Food supply is generally acknowledged to be a major environmental factor influencing the timing of, and level of investment in, breeding by birds (for a review, see Martin (1987)). Correlative studies have often found a positive relationship between natural food availability and reproductive parameters, such as date of laying and clutch size (e.g. Perrins 1991). Evidence for a causal relationship between food supply and reproductive output has been sought in a wide variety of experimental studies, where wild birds have had their natural diet supplemented by the provision of additional food. The results of these studies have, however, been equivocal, and generally show that, although enhanced food supply often results in an advancement of laying date, only sporadically is there any effect on the size or number of eggs laid (see reviews in Martin (1987), Arcese & Smith (1988), Boutin (1990) and Aparicio (1994)). Most of these studies have implicitly assumed that it is the energy content of food that limits egg production; in contrast, little attention has been paid to food quality. However, Jones & Ward (1976), Ewald & Rohwer (1982), Arcese & Smith (1988) and Bolton *et al.* (1992) have suggested that birds may have requirements for specific nutrients during egg production, and that limited availability of these in the diet may constrain egg production. If this is the case, then results of supplementary feeding experiments may depend to a large extent on the ability of the supplementary diet to provide the limiting nutrient(s). Bolton *et al.* (1992) supplemented the diet of lesser black-backed gulls (*Larus fuscus*) with either fat, fish or eggs, all foods being provided in quantities of equivalent calorific value. They demonstrated that the energy content of the food did not influence the size of eggs laid, but fish- and egg-fed birds both produced larger eggs than control or fat-fed birds, those receiving the egg supplement being the largest. The authors proposed that this result may be due to differing protein quality between diets. Egg proteins in birds differ in their composition from many plant and animal proteins, and egg production could be limited not by overall protein availability, but by the availability of specific essential amino acids (Houston *et al.* 1995*a*; Selman & Houston 1996).

We have previously conducted trials with blue tits (*Parus caeruleus*) in which birds were given supplementary diets of either fat or a diet containing eggs, and these showed that whereas the timing of laying was influenced by dietary energy supply, egg production was constrained by the availability of other nutrients (Ramsay & Houston 1997): only those birds fed a supplementary diet containing eggs showed increased egg production. It is possible that protein quality was the limiting factor in this case, and in Bolton *et al.*'s (1992) study on gulls, but we do not know this because the supplement contained entire eggs and so many other nutrients could also have been involved. Here we report the findings of an experiment in which blue tits were provisioned with diets that differed only in amino acid composition. One group was given a supplementary diet of ground peanuts augmented with five amino acids in order to bring the essential/semi-essential amino acid balance close to that found in egg proteins. We selected those essential amino acids that were present at higher concentration in egg protein than peanuts and that are also known to result in enhanced egg production in poultry (Scott *et al.* 1982). A second group were given an identical peanut diet containing sufficient glutamic acid to make the two supplementary diets isonitrogenous. The two supplementary diets were therefore of identical calorific, 'crude' protein and non-protein nutrient content. A third control group received

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Table 1. *Amino acid composition of foods* (*mg g^{-1} nitrogen*)

(All values obtained directly, or calculated from Paul & Southgate (1978) and Paul *et al.* (1978).)

	essential										semi-essential		non-essential					
	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Arg	His	Cys	Tyr	Ala	Asp	Glu	Gly	Pro	Ser
whole raw hens' eggs	350	520	390	200	320	320	110	470	380	150	110	250	340	670	750	190	240	490
HQP	178	338	390	200	263	321	111	219	591	128	111	203	203	600	962	296	229	253
LQP	178	338	186	60	263	135	60	219	591	128	68	203	203	600	1667	296	229	253

no food supplement. To our knowledge, the results provide the first unequivocal evidence that the investment in eggs by wild birds may be constrained by the availability of particular amino acids in the diet.

2. MATERIALS AND METHODS

(a) *Study site*

Trials were carried out in 1995 on blue tits using nestboxes in an oak-dominated woodland around the University Field Station, Rowardennan, Loch Lomond, 30 km North of Glasgow. Details of the study area are given in Ramsay & Houston (1997).

(b) *Supplementary diets*

Finely ground peanuts were used as the base for both supplementary diets. To produce the 'high quality protein' (HQP) diet, the essential amino acids, methionine, lysine, cystine, threonine and tryptophan were added to the peanut base in similar proportions (mg of amino acid per g of nitrogen) as found in hens' eggs (Paul & Southgate 1978; Paul *et al.* 1978). The amino acid composition of egg proteins is relatively invariant between bird species (Murphy 1994), and tits' eggs are similar in amino acid balance to those of poultry (Ramsay 1997). High purity, l-isomer, crystalline amino acids were used to supply the cystine, threonine and tryptophan; 2.3 g Cys, 9.9 g Thr and 2.7 g Trp being added per 1 kg of ground peanuts. L-lysine hydrochloride was used to supply the additional lysine. L-lysine hydrochloride has a natural L-lysine potency of 78.4% (Scott *et al.* 1982). Therefore, 13.9 g of L-lysine hydrochloride was added to provide 10.9 g of extra available Lys per 1 kg ground peanuts. Similarly, DL-methionine has a natural L-methionine potency of 78–92% (Scott *et al.* 1982); we assumed a value of 85%. Therefore, we added 8.8 g of DL-methionine to provide 7.5 g of extra available Met per 1 kg of ground peanuts.

In the 'low-quality protein' (LQP) diet, glutamic acid (a non-essential amino acid, not likely to be limiting) was added to the peanut base to produce a mixture with the same total nitrogen content as the 'high-quality protein' mixture (37.6 g Glu per 1 kg of ground peanuts). The amino acid compositions of the supplementary diets are shown in table 1.

(c) *Feeding schedule*

All nestboxes were checked weekly for signs of nestbuilding, beginning in late March. When a new nest was found, which was at least one-quarter built, it was randomly assigned to either one of the experimental groups or to the control group. Supplementary food was provided for experimental groups from the following day, in wiremesh feeders suspended within 1 m of the chosen nestbox. Experimental birds were observed to begin using feeders shortly after they were deployed. Feeders were replenished regularly, so that supplementary food was provided *ad libitum*. Feeding continued until clutch completion. Control birds received no additional food. Blue tits become territorial many weeks before laying and continue territoriality until chick rearing (Perrins 1979). Supplementary feeding at a nestbox began, on average, some 2–3 weeks before the onset of laying, and observations suggested that tits from neighbouring territories were denied access to the feeders, which were used exclusively by the intended nesting pair.

(d) *Assessment of reproductive parameters*

Once a nest was fully built, it was checked daily to determine the onset of laying. After the first egg was laid, a nest was visited every second day until clutch completion. At each visit, any new eggs were numbered using permanent, non-toxic ink pens, weighed (to 0.01 g) with an Acculab Pocket Pro portable electronic balance and their lengths and widths measured (to 0.05 mm) with vernier callipers. Egg volume was calculated using the equation: $\text{egg volume} = 0.51 \times \text{egg length} \times \text{egg width}^2$ (from Hoyt 1979).

If a female had not begun incubation by clutch completion, the nest continued to be checked daily to determine the onset of incubation. Nests were then not visited for 10 d, after which they were visited daily to determine the hatching date of all the viable eggs. At each visit, any new hatchlings were weighed (to 0.01 g). The duration of incubation was calculated as the time in days from determined onset of incubation to hatching of the first chick. Nests were visited again when the chicks were 7 and 14 days old for weighing and measurement of wing and tarsus length. Blue tits fledge at *ca.* 18 d; therefore nests were visited at *ca.* 25 d after hatching to check if all young had fledged.

3. RESULTS

The onset of laying in the two fed groups was slightly earlier than in the control group (mean date in days after 29 April ± s.e. (n): HQP, 6.0 ± 1.2 (20); LQP, 4.8 ± 1.0 (18); control, 8.4 ± 1.2 (20)) although this was not significant ($F = 2.47$, d.f. = 2,55, $p = 0.09$). The eggs within a clutch are not statistically independent (variation in egg size and weight was considerably greater between clutches than within; F-test, $p < 0.00005$ in all cases) and, therefore, analyses of egg size and weight between feeding treatments were carried out on clutch means. There were no significant differences in egg volume (mean egg volume (mm^3) ± s.e. (n): HQP, 1080 ± 20 (20); LQP, 1085 ± 19 (18); control, 1051 ± 19 (20); $F_{2,55} = 0.93$, n.s.) or egg mass (mean egg mass (g) ± s.e. (n): HQP, 1.139 ± 0.021 (20); LQP, 1.155 ± 0.021 (18); control, 1.113 ± 0.017 (20); $F_{2,55} = 1.14$, n.s.) between experimental and control groups.

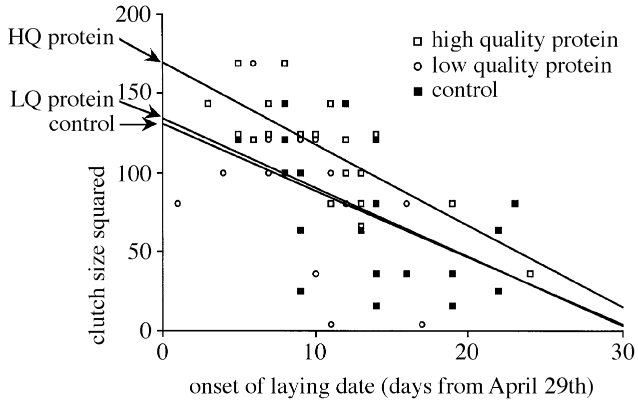

Figure 1. Regressions of clutch size against laying date for birds fed supplementary diets of high-quality ($y=168.69 - 5.13x$; $r=0.79$, $p<0.0001$) and low-quality ($y=134.45 - 4.37x$; $r=0.42$, $p<0.8$) protein, and control birds receiving none ($y=131.49 - 4.25x$; $r=0.53$, $p<0.02$).

Table 2. *Mean (±s.e. (n)) clutch size in control and supplementary fed birds*

(Calculated using square-transformed data. Values presented have been back-transformed.)

	HQP	LQP	control
clutch size	10.58	9.56	8.65
s.e.	+0.35 − 0.37,	+0.50 − 0.56,	+0.52 − 0.60
n	(20)	(18)	(20)
clutch size adjusted for laying date	10.35	8.68	8.77

Clutch size data were transformed by squaring, in order to normalize them, before performing parametric analysis. Clutch size declined significantly through the season. To control for this, an analysis of covariance was carried out with laying date as a covariate. After controlling for laying date, there was no difference in mean clutch size between the LQP group and the control group, whereas the HQP group showed a significantly greater mean clutch size than either of the other two groups (figure 1 and table 2; effect of laying date, $F_{1,57}=26.36$, $p<0.0005$; effect of treatment, $F_{2,55}=4.15$, $p<0.025$). Birds with access to a high-quality protein diet produced, on average, about 1.6 additional eggs in each clutch compared with control pairs.

There were no significant differences between the treatment groups in onset of incubation, incubation duration, hatching date, hatching success, chick mass or chick size (Ramsay 1997). Brood sizes were not significantly different between groups, although there was a consistent trend for more chicks to be present in broods from HQP females at hatching (mean ± s.e. (*n*): HQP, 8.56 ± 0.47 (18); LQP, 7.86 ± 0.43 (14); control, 7.36 ± 0.88 (11); $F_{2,40}=1.11$, n.s.), at 7 days (mean ± s.e. (*n*): HQP, 8.27 ± 0.60 (15); LQP, 7.62 ± 0.40 (13); control, 7.56 ± 0.90 (9); $F_{2,34}=0.44$, n.s.), and at 14 days (mean ± s.e. (*n*): HQP, 8.20 ± 0.60 (15); LQP, 7.54 ± 0.39 (13); control, 7.33 ± 0.91 (9); $F_{2,34}=0.56$, n.s.).

4. DISCUSSION

Egg production is a demanding process for a bird and a female blue tit can lay a clutch of eggs weighing more than her own body mass (Perrins 1979). The estimated peak daily energy requirement for egg production ranges from 13 to 216% of basal metabolic rate for different bird species (Walsberg 1983; Ricklefs 1974). Robbins (1981, 1993), however, has pointed out that the estimated increase in daily protein requirement for egg production varies from 72 to 237% of maintenance requirements for different avian groups. The emphasis that has been placed in many previous experimental studies on energetic limits to egg production in birds may, therefore, have overlooked a potential protein limitation. Egg proteins contain unusually high levels of sulphur amino acids, and in domestic poultry, egg production is enhanced if the diet is enriched with these and some other essential amino acids (Scott *et al.* 1982). If the diet available to a bird is deficient in some of the essential amino acids required for egg production, it may obtain these from endogenous sources: many bird species show significant depletion of muscle at the time of egg formation (Houston *et al.* 1995*b*) and muscle proteins can contribute directly to egg formation (Houston *et al.* 1995*c*). Further, the quality of the endogenous protein established by a female bird before the breeding season can have a marked effect on her subsequent egg production (Selman & Houston 1996). Research on blue tits, however, has revealed no significant loss of female muscle tissue during the egg-laying period (Woodburn & Perrins 1997). Presumably, therefore, in this species dietary intake has to provide all the nutrients used for egg formation. It is well known that birds which feed on low protein diets, such as seed-eating zebra finches, *Taeniopygia guttata*, can greatly increase egg production when the protein content of the diet is increased (Selman & Houston 1996; Houston 1997; Williams 1997). It is, however, remarkable that species which are apparently feeding on high-quality animal protein diets, such as gulls (Bolton *et al.* 1992) and tits (Ramsay & Houston 1997), should be constrained by protein availability. The estimated daily energy expenditure of a laying blue tit (approximately 69 kJ d^{-1}) would require the consumption of about 3580 mg dry mass of small caterpillars (Ramsay 1997). However, amino acid analysis of caterpillars has shown that this mass would contain more than four times the estimated daily requirement for even the most potentially limiting amino acid during egg production (Ramsay 1997). Murphy (1994) carried out similar calculations for a model insectivorous passerine, and also concluded that it was unlikely that amino acids could limit egg production because the level of food intake necessary to satisfy energy requirements would always exceed the minimum requirements for all amino acids. The results presented here, however, do suggest that the amino acid composition of the diet is limiting. There are a number of reasons why estimates based on simplistic consumption figures may be incorrect and why even an insectivorous passerine such as the blue tit might face limitations imposed by protein quality.

First, it is incorrect to assume that tits feed exclusively on an insect diet around the time of egg production. Caterpillars are not composed only of animal tissue and up to 20% of their body mass is contributed by vegetation within the gut (Ramsay 1997). In addition, tits alter their diet in early spring. Betts (1955) studied parid diets in oak woodland by gizzard analysis throughout the year. In March and April, all birds examined ($n=26$) had over 40% oak bud tissue in the gizzard, and over two-thirds of these birds contained over 75% plant material, by volume. Such quantities were not found at any other time of year. Insect abundance is extremely low at the time of egg laying. Birds may be seeking insect larvae within oak buds, and swallow the surrounding bud tissue unintentionally. However, tits are so dextrous in the manipulation of prey with the bill that it is difficult to believe they could not isolate an insect grub more efficiently. Perhaps leaf buds are deliberately eaten at this time of year because the cells have thin, easily ruptured walls and are relatively rich in lipid- and carbohydrate-containing protoplasm (Esau 1977; Berggren 1985). There are also many records of blue tits drinking the sugar-rich sap of trees and nectar from flowers at this time (Cramp & Perrins 1993). Additional pressure to feed on high-energy, but low-protein quality, plant foods may be caused by the high calcium demand for eggshell formation. The availability of calcareous items at our study site was extremely low (Ramsay & Houston 1998) and in this situation tits have to spend a considerable period of time each day foraging for calcium-rich prey (Graveland & Berends 1997). Some selective foraging on readily accessible high-energy food items may be required to fuel this search for calcium.

Second, calculations of the dietary amino acid requirements for egg production require estimates of the efficiency of protein digestion, nutrient absorption and the conversion efficiency into egg proteins. Virtually nothing is known about these processes in the tit, the only data available for birds coming from domestic poultry, which may not be applicable to insectivorous passerines. Even if we knew likely parameters for an insectivorous bird, there are likely to be substantial changes to digestive physiology at the time of laying. For example, it is known that gut morphology of tits changes at this season (Perrins 1979), that around laying time the gut may contain a substantial quantity of bulky plant material, and that digested calcium ion concentration is many hundreds of times greater than normal owing to snail shell consumption to allow eggshell formation. All of these factors are likely to influence digestive efficiency and nutrient absorption.

The influence of food quality on parid breeding has also been examined experimentally by Nager *et al.* (1997) and Svensson & Nilsson (1995), neither of whom found any evidence for a non-energetic nutritional constraint. Svensson & Nilsson provided some birds with mealworms and egg proteins, but their sample size was small ($n=7$) and the Nager *et al.* study used mealworms, which may not provide the limiting nutrients. In addition, both of these studies were carried out in mixed woodlands. Oak is very late in coming into leaf, and arthropod abundance is closely related to arboreal bud burst. Tits breeding in mixed woodlands may have access to insects feeding on other, earlier emerging tree species, and therefore not be so nutritionally constrained as tits breeding in an oak-dominated woodland. Differences in habitat quality are known to affect egg production in tits (Perrins 1979; Isenmann 1987; Svensson & Nilsson 1995; Nager *et al.* 1997).

We conclude, therefore, that, although it initially seems improbable that an insectivorous bird species might face nutritional limitations on egg production as a result of amino acid imbalance, this could be a previously neglected proximate constraint on egg size or number. Further, even within parids, nutritional constraints on egg production may vary as a result of differences in habitat type.

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