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Egg development and the related nutrient reserve depletion in the pied flycatcher, Ficedula hypoleuca

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Pied flycatcher, Ficedula hypoleuca (Pallas) females (N=25) were collected in different breeding phases (pre-breeding, pre-laying, laying, incubating, feeding young) in the Oulu region of northern Finland. The energy investment rate for eggs and the lipid and protein reserves (measured as ash-free lean dry weight) of all females were determined.

The follicle growth period was slightly over 4 days, during which investments were successively 4, 15, 32, and 34 % of final yolk weight. The albumen, shell and the rest of the yolk (15 %) were produced during the last 24 h. The modal clutch of 6 eggs contains about 0.6 g lipid, 1.5 g protein and 0.3 g calcium carbonate (measured as shell ash), which amounts are invested in the eggs during 10 days. The maximum investment rate (2.2 kcal/d, measured as egg content energy) occurred only during two days of the cycle, the other days reaching 2 to 98 % of the maximum rate. Pied flycatcher females lost 0.7 g of lipids but only a very small amount of proteins from the body reserves during laying. The lipids were presumably used not only to cover the investments in yolk, but also for fueling the search for protein-rich food. Pied flycatchers thus rely mainly on foraging to cover the protein demands for their eggs.

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1. Introduction

Egg production is an energetically demanding period for female birds (King 1973, Ricklefs 1974). The growth of yolk takes place during a period lasting 3-4 days in small passerine birds and 6-10 days in large birds up to the size of the domestic hen or the herring gull, *Larus argentatus* (King 1973). Albumen is secreted and shell deposited during the last 24 hours before the eggs are laid (e.g. Romanoff & Romanoff 1949). By extending the period of formation of one egg over several days, females decrease the daily costs of producing a clutch (King 1973). As the daily energy expenditure may thus be lowered, the birds are able to lay relatively early in the season.

To meet the heavy investment period of egg production, females often start to invest in the reproductive organs and collect nutrient reserves into their body far before laying commences. In the house sparrow, *Passer domesticus,* the ovary begins to increase in size about 60 days before ovulation (Pinowska 1979) and this species, as also the red-billed quelea, *Quelea quelea* (Jones & Ward 1976, 1979), stores protein and fat reserves. Other strategies to meet nutrient demands consist of increasing the quality and quantity of food eaten (brown-headed cowbird, *Molothrus ater,* Ankney & Scott 1980), or of collecting fat reserves and eating a high-protein diet (pintail, *Anas acuta,* Krapu 1974).

In this study the energy investment into the eggs and the rôle of fat and protein reserves for egg production in the pied flycatcher were determined. In this paper the daily growth rate of eggs and the female's ability to store and deplete nutrient reserves during the laying of a clutch are discussed. Other aspects of nutrient reserve dynamics in the breeding season will be treated in a later paper.

2. Material and methods

A total of 25 pied flycatcher females were collected from Oulu (65°N, 25°30′E) and Siikajoki (64°50′N, 24°50′E) in the summers of 1979-1981 (Table 1). Females of known breeding history were collected in different phases of breeding: pre-breeding birds were those collected as late migrants at the Tauvo Bird Observatory, pre-laying ones were captured with mist-nets near nest-boxes containing a finished nest-cup; laying or incubating females or those feeding young were taken from nest-boxes.

After killing by thoracic pressure the birds were put into a cold-box (c. -20°C) and transported to the laboratory where they were weighed and kept in a deep-freezer until preparation. After thawing, the stomach contents were removed and subtracted from the total weight. The feathers were removed and weighed. The body was then dissected into the following components: ovary, oviduct, breast muscles (bilaterally, including the *pectoralis, supracoracoideus* and *coracobrachialis*), liver and the rest of the body. The diameter of the four largest follicles (half-thawed) was estimated from the ovaries of laying-stage birds, after which the follicles were separated and thereafter treated as the other body components. The follicle sizes of pre-breeding and pre-laying females also allowed the determination of the reproductive phase of these birds (see Payne 1969, Jones & Ward 1976). The oviducal eggs, together with those laid, were weighed and after cracking, the yolk and the albumen plus the shell were poured into small preweighed cups. The egg components were treated according to the procedures described in Ojanen (1983a) for obtaining caloric values for these two subcomponents.

All dissected components of the body were weighed, dried at 80°C until they were of constant weight, and then reweighed. The water fraction was given by the difference between these two weighings. The lipids were extracted from the dry material with a mixture of 5:1 petroleum ether and chloroform (Ricklefs 1976). The lipid weight was calculated as being the difference between the weights before and after extraction. The lean dry matter was then ashed in a muffle furnace at 600°C for obtaining the proportions of ash and ash-free lean dry material. The usual combustion temperature of 550°C was too low for our particular furnace. The chemical constituents were converted to energy equivalents by using the following coefficients: lipid = 9.5 kcal/g and protein = 5.65 kcal/g (Ricklefs 1974). In calculations, the ash-free lean dry material was assumed to consist of protein. Thus, the protein component includes a small amount of carbohydrates (4.2 kcal/g) which causes a slight overestimation of the caloric content of tissues.

Multiplying the values, expressed in kilocalories, by a factor of 4.187, gives SI-units of kilojoules.

3. Results

3.1. Size, growth rate and composition of follicles

In laying birds the size of the four largest follicles averaged 9, 6, 4, and 2.5 mm in diameter. The numerous other follicles were smaller than 2 mm in diameter. One laying female had only two large follicles exceeding 2 mm in size, and only these were collected. The status of pre-laying females was judged from the size of the largest follicle in the ovary.

The weight and composition of the four largest follicles in laying birds are given in Table 2. The

Table 1. The dates of collection, the mean body weights and phases of breeding of female pied flycatchers collected from the Oulu area or from Siikajoki (migrating individuals). See also text.

	Migrating	Pre-laying	Laying	Incubating Early	Late	Feeding young Early	Late
Collection dates	3-4.6.1980 23-30.5.1981	4.6.1980 30.5.1981	4-5.6.1979	5.6.1979	12.6.1981	22.6.1981	30.6.1981
N	7	3	6	3	3	3	3
Body weight g	13.6±0.50	15.8±1.51	16.1±1.06	14.3±0.58	14.3±0.83	12.9±0.50	12.9±0.19
Number of eggs or young in the nest	-	-	1-3	6-7	5-7	5-7	2-5
Incubation lasted (days)	-	-	-	1-3	9-10	-	-
Age of young (days)	-	-	-	-	-	4-5	10-13

Table 2. Weight, composition and energy contents of the large developing follicles and the rest of the ovary in the pied flycatcher (mean± *SD*).

	Follicles[1] I	II	III	IV	The rest of the ovary[2]
N	6	6	5	5	
Weight mg	315 ±26	187 ±21	68 ±23	14±13	66 ±17
% water	48.6±1.2	50.1±1.3	53.6±1.3	66.6±9.1	78.3±1.4
% lipid	32.8±1.4	32.3±0.4	29.8±1.1	15.2±5.0	4.6±2.2
% lean ash-free dry	17.5±1.2	16.7±1.1	16.0±1.4	18.0±8.5	16.5±1.4
% ash	1.2±0.3	1.0±0.3	0.9±0.6	trace	0.6±0.6
Per cent of final yolk size	85.0±7.4	50.4±6.2	18.8±6.8	3.9±3.5	
Kcal energy in lipid	0.98±0.11	0.57±0.07	0.19±0.07	0.02±0.03	0.03±0.02
Kcal energy in lean ash-free dry	0.31±0.03	0.17±0.02	0.06±0.02	0.01±0.01	0.06±0.02
Kcal total energy	1.29±0.12	0.74±0.09	0.25±0.09	0.03±0.04	0.09±0.03
% energy of total ovary (2.40 Kcal)	54±5	31±4	10±4	1±2	4±1

[1]The largest follicle was numbered as I, the smallest of the four largest as IV; others had a diameter of less than 2 mm and were not collected. One female had only two follicles larger than 2 mm in diameter.

[2]Excluding the large follicles of diameter greater than 2 mm.

follicles began to increase in size four days before ovulation, weighing at that stage 4 % of the final size. The weight gain was 15, 32, and 34 % of the final size during the three consecutive days. At the time fo collection, about 6 o'clock in the morning, the largest of the follicles had reached about 85 % of the final weight (Table 2). The proportion of lipids increased as the follicles became larger in size and concomitantly the proportion of water decreased. The proportion of proteins (lean ash-free matter) was unchanged, as was the proportion of ash. The largest follicle had about half of the energy content of the ovary. Excluding the four largest follicles, the rest of the ovary contained about 4 % of the total energy of the ovary.

3.2. Energy investments in the eggs

The energy contents of an average egg of laying-stage pied flycatchers was 2.21 ± 0.17 kcal (*SD*; *N*=6, inclusive of shell energy). The investment of energy in one egg took place during five days. During the first four days the major portion of the yolk was deposited and during the last 24 hours the rest of the yolk (15 %), albumen and shell were formed, these constituting about 42 % of the total energy investment (Fig. 1). Interestingly, the largest follicles reached only 85 % of weight, but nearly 100 % of the energy content of the yolk of the eggs of these females. Thus, the estimated energy proportions for the yolk, albumen and shell presented in Fig. 1. differ

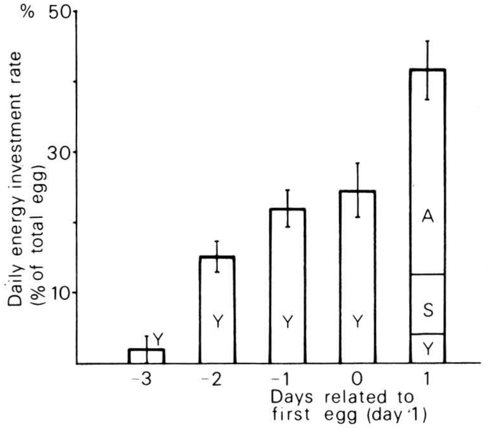

Fig. 1. Investment rate (kcal/day, % of total) of energy of yolk (Y), albumen (A) and shell (S) in one egg of pied flycatcher. Vertical bars denote 2 *SD*, number of females studied = 6. Note that the investment of day 1 includes that proportion which is invested between collecting time (day 0, 6 a.m.) and laying (day 1, 6–8 a.m.).

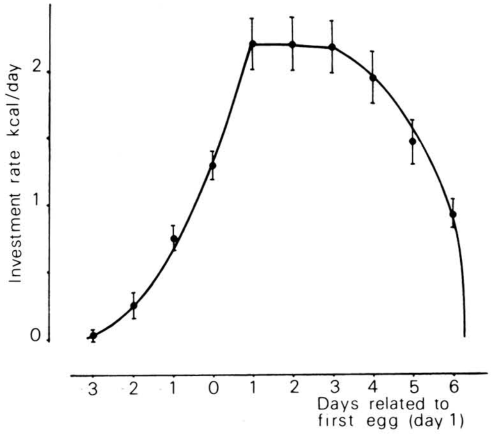

Fig. 2. Total investment for eggs (kcal/day) in pied flycatcher during a laying cycle of 6 eggs. Vertical bars denote 2 *SD*, number of females studied = 6.

slightly from the values given by Ojanen elsewhere (1983a).

The total energy of 6 eggs, the most common clutch-size in the Oulu area (Ojanen et al. 1978), averaged 13.26 kcal, which was invested within 10 days (Fig. 2). The heaviest investments (2.21 kcal per day, i.e. the energy content of one egg, see also King 1973) took place during two days only of the investment period, while during eight other days investments varied form 98.2 to 1.8 % of this maximum value.

3.3. Changes in the components and energy contents of the body during laying.

Females in the laying stage weighed 16.6 ± 1.1 g, part of the variation being due to the presence or absence of the oviducal egg (Table 1). Pre-laying birds weighed 0.6 g, and incubating birds 2.3 g, less than the laying ones, the latter value being significantly smaller than that of the layers (*t*-test one-tailed, $P<0.05$). The estimated diurnal weight cycle during laying was from 15.58 ± 0.283 g (*N*=6), this reflecting the weight just after laying of the egg, to at least 17.17 ± 0.302 g, this being the pre-laying weight and assuming the differences to equal the weight of an average egg from these females (1.62 ± 0.068 g, *N*=6).

The weight increase of the ovary and oviduct from the pre-laying to laying was modest (0.5 g), and a significant weight reduction in all components was noted when the females started to incubate (Table 3). The ovary of a laying female had 2.40 kcal energy, of which 75 % was in lipid depots. The energy content of the ovary

Table 3. Body components (g, mean±*SD*) of pied flycatchers at different laying stages of the female and statistical significancies of *t*-tests between successive groups (P_1 and P_2) and pre-laying and incubating females (P_3).

	Pre-laying	P_1	Laying	P_2	Incubating	P_3
Ovary						
weight	0.41±0.30	-	0.67±0.07	***	0.04±0.01	-
lipid	0.11±0.08	-	0.19±0.02	***	0.00±0.00	-
water	0.23±0.15	-	0.37±0.05	***	0.03±0.01	-
lean dry	0.08±0.06	-	0.12±0.01	***	0.01±0.00	-
Oviduct						
weight	0.73±0.46	-	1.00±0.07	***	0.18±0.05	-
lipid	0.01±0.00	-	0.01±0.00	*	0.01±0.00	-
water	0.54±0.34	-	0.75±0.07	***	0.14±0.03	-
lean dry	0.18±0.12	-	0.24±0.01	***	0.04±0.01	-
Breast muscle						
weight	2.27±0.03	-	2.28±0.10	-	2.33±0.10	-
lipid	0.09±0.02	-	0.06±0.02	-	0.05±0.00	*
water	1.60±0.05	-	1.66±0.07	-	1.70±0.07	-
lean dry	0.58±0.02	-	0.56±0.04	-	0.59±0.03	-
Total body[1]						
weight	12.37±0.32	-	12.48±0.30	-	12.30±0.57	-
dry weight	4.61±0.48	-	3.99±0.25	-	3.69±0.28	-
lipid	1.59±0.56	-	1.14±0.30	-	0.87±0.16	o
water	7.76±0.42	*	8.51±0.11	-	8.62±0.35	o
lean dry	3.02±0.12	*	2.85±0.07	-	2.82±0.15	-
ash	0.49±0.05	-	0.44±0.02	*	0.37±0.03	*
N	3		6		3	

[1]Body weight minus weights of reproductive organs and feathers

dropped by 98 % as incubation commenced, and the respective decrease in the energy content of the oviduct was 82 % from laying to start of incubation (Table 4).

The fresh and lean dry weights of breast muscles remained unchanged from the pre-laying to the incubating phase (Table 3). The amount of water in the muscle increased slightly as laying progressed and incubation started. The amounts of lipids in the breast muscle was greatest in pre-laying birds and it decreased towards the incubating stage. Also, the energy contents of breast muscle tended to decrease as laying progressed. This was due to the decrease in lipids, not in lean dry matter (Table 4). The lipid stores in the body (excluding the ovary and oviduct) were 1.59 g in the pre-laying females, when the amount of lean dry matter also tended to be high (Table 3). Interestingly, in migrating females there were 1.90 ± 0.52 g lipids (*N*=7), but the weight of lean dry matter was at the same level as in laying birds. The amount of lipids tended to decrease steadily when breeding advanced: the incubating females had only half of the value of pre-laying birds (Table 3) and in those feeding the young even smaller amounts were recorded (0.63 ± 0.16 g, *N*=6). While the amount of lipids showed a significant decreasing trend as breeding advanced, the amounts of lean dry matter fluctuated only slightly. Values for the latter decreased from the pre-laying to the laying stage, but otherwise very permanent levels were maintained during all stages of breeding including the pre-breeding and feeding phases (levels being about 2.8 - 2.9 g lean dry matter). The amount of ash in the total body decreased significantly by 24 % from the pre-laying to the incubation phase (Table 3). The energy in lipids dropped by nearly 7 kcal (45 %) from pre-laying to incubating (Table 4) (by 9.7 kcal or by 54 % from the pre-breeding phase!). The decrease in the total energy, amounting to about 7 kcal, was mainly due to the decrease in lipid level, and owing to large individual variations was only weakly significant (*P*<0.10).

4. Discussion

4.1. Energetics of egg formation

Silverin (1980) observed that the recrudescence of the ovary has already begun when female pied flycatchers arrive in Sweden at the beginning of May, about two weeks before laying. Its weight averaged 15 mg, c. 2 % of the estimated maximum. The energy needed for this growth comprises about 2-13 % of basal metabolic rate,

Table 4. Energy contents (Kcal, mean±*SD*) of different body parts of pied flycatchers, collected at different stages of laying and statistical significancies of *t*-tests between successive groups (P_1 and P_2) and pre-laying and incubating females (P_3).

	Pre-laying	P_1	Laying	P_2	Incubating	P_3
Ovary						
lipid	1.03±0.80	-	1.79±0.22	***	0.01±0.00	o
protein	0.40±0.31	-	0.61±0.08	***	0.04±0.01	-
total	1.44±1.11	-	2.40±0.29	***	0.05±0.01	o
Oviduct						
lipid	0.12±0.04	-	0.09±0.01	**	0.06±0.01	o
protein	0.95±0.66	-	1.28±0.06	***	0.20±0.06	-
total	1.07±0.70	-	1.37±0.05	***	0.25±0.08	-
Breast muscle						
lipid	0.89±0.21	o	0.57±0.18	-	0.43±0.03	*
protein	3.13±0.11	-	3.05±0.20	-	3.13±0.14	-
total	4.01±0.19	*	3.62±0.25	-	3.56±0.17	*
Total body[1]						
lipid	15.09±5.31	-	10.86±2.87	-	8.30±1.50	o
protein	14.31±0.41	*	13.62±0.35	-	13.81±0.69	-
total	29.40±5.00	-	24.48±2.60	-	22.10±1.97	o
N	3		6		3	

Note 1; see Table 3.

BMR, in different bird species, and usually the time of rapid growth is extended over a period of days, as in the pied flycatcher, so that it does not cause any stress on the individuals' metabolic capacities (see also Ricklefs 1974).

The follicular growth rate is commonly estimated by weighing the developing follicles and assuming that the difference in the weights of successive follicles equals the daily increment of growth (e.g. Payne 1969). The weight increase of the follicles does not exactly fit the rate of increase in its energy contents, as the smaller ova contained a greater proportion of water and proteins than the larger ones (Table 2), a fact also reported for the domestic hen (Romanoff 1943), for example. Thus, the increase in the energy content of the follicle was used to represent the investment rate.

The rapid growth phase of the follicle has been estimated to last from about 2–3 to 5 days in various passerine species (Nice 1937, Kluijver 1951, Payne 1969, Kern 1970 (cited in King 1973), Ricklefs 1974, Pinowska 1979). Hence, the observed rate of 4 days in the pied flycatcher is intermediate. The difference in the time required for the rapid growth of the follicle in these species is perhaps an adaptation to the predictable availability of protein-rich food in spring.

The maximum daily energy requirements for egg formation were observed in the pied flycatcher only during two days of the period of the full clutch production of 6 eggs (Fig. 2). As King (1973) pointed out, the maximum rate of investment is attained only when the clutch size is 5 eggs or more, the laying interval between the eggs is one day and the yolk is deposited over four days. When the clutch size is 4 eggs or smaller, or the laying interval longer than 1 egg per day, the relative maximum rate of energy demand remains lower. On the other hand, clutches of more than 5 eggs increase the energy stress on the female only slightly more than clutches of 5 eggs or smaller, as the maximum investment rate has already been attained.

The energy burden of egg laying can be expressed as a fraction of BMR. This shows the average amount of productive energy (available minus existence energy, see West 1960, Kendeigh et al. 1977), which a bird can invest in the eggs daily. In a number of passerine species this rate is within the range of 35–60 % of BMR (Table 5). In the bulk of these species the fraction is about 35–45 %, while that of the pied flycatcher is markedly higher, being about 60 %.

The strategy of having several simultaneously developing eggs is adaptive for the birds, as it allows the female to form the clutch during a relatively short period. During adverse weather this habit also allows females which have started to lay to lengthen the laying period and reduce the daily investments by interrupting laying for one or more days. A one-day pause in laying reduces the total investment in such a way that sub-maximal investments occur during the 4–5 following days (Fig. 3). Field observations have revealed that one-day pauses in laying are the

Table 5. Estimates of the caloric cost of producing eggs and of the peak caloric cost (Kcal/egg/BMR) during the laying period in passerines with a laying interval of one day between eggs of a clutch.

Species	Body weight g	Mean clutch size	Mean egg weight g	Kcal/ egg[1]	Follicle growth period d	Kcal/ clutch	BMR[2]	Kcal/egg/ BMR (%)	Source
Starling	76	5.29	7.2	10.63	3	56.02	17.67	60.2	1, 2
House sparrow	31	5.43	2.7	4.38	5	23.78	9.22	45.7	3, 4, 5, 6
White-crowned sparrow	27	4.7	2.5	3.75	?	17.63	8.34	44.0[3]	7
Great tit	18	9.24	1.58	2.86	3–4	26.43	6.21	46.1	8, 9, 10
Redstart	16	6.67	1.90	3.33	?	22.21	5.70	58.5	3, 9, 11, 12
Pied flycatcher	14	6.33	1.67	3.17	4	20.07	5.18	61.2	9, 10, 13
Zebra finch	12.6	3.88	0.97	1.70	?	6.60	4.79	35.5[3]	14
Willow tit	11	7.84	1.35	2.02	?	15.84	4.34	46.5	4, 15
House wren	11.5	6.4	1.45	2.11	?	13.50	4.49	47.0	16
Long-billed marsh-wren	9.4	4.50	1.17	1.76	?	7.92	3.88	45.4[3]	17

[1] Productive energy assuming energy efficiency as 70 % (King 1973)
[2] Calculated for rest period after Aschoff & Pohl 1970
[3] Value perhaps too large due to small clutch size (<5, see also Ricklefs 1974)

Sources: 1) Ricklefs 1974; 2) Ojanen et al. 1979 (clutch size); 3) Own unpubl. data (female weight); 4) Alatalo 1978 (clutch size); 5) Tangl 1903 (egg weight and energy content); 6) Pinowska 1979 (follicle growth period); 7) King et al. 1966; 8) Orell 1976 (female weight); 9) Ojanen et al. 1978 (clutch size); 10) Ojanen 1983a (egg weight and energy content); 11) Schönwetter 1972 (egg weight); 12) Carey et al. 1980 and Ar & Yom-Tov 1978 (egg energy content, partly recalculated); 13) Ojanen 1983b (female weight); 14) El-Wailly 1966; 15) Own unpubl. data (female weight, egg weight and energy content); 16) Kendeigh et al. 1956; 17) Kale 1965.

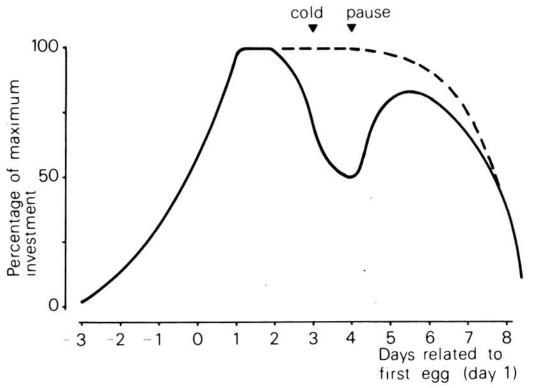

Fig. 3. Hypothetical curve showing the effect of a one-day pause in the laying cycle, caused by cold weather, on the daily energy investment for eggs in a 7-egg clutch. The stripped line represents the daily invetsments without any pauses (in an 8-egg clutch !), the area between this and the solid line the "saved" energy (of one egg) divided over five days.

most common during adverse weather (e.g. Winkel & Winkel 1974), just as presumed by the model.

4.2. Protein reserves during laying

Protein reserves occur throughout the sceletal muscles and other body tissues of birds, especially the breast muscles (e.g. Hanson 1962, Kendall et al. 1973, Jones & Ward 1976, Ankney & MacInnes 1978, Fogden & Fogden 1979, Ankney & Scott 1980). In this paper the lean dry weight of the breast muscle and the lean dry weight of the body (excluding reproductive organs and feathers) were used as indexes for the protein stores (see also Jones & Ward 1976, Fogden & Fogden 1979, Pinowska 1979).

In the pied flycatchers studied an average egg contained 216 mg protein — of which 56 mg was in yolk, 113 mg in albumen and 47 mg in shell — 103 mg lipids and 48 mg shell ash (Ojanen 1983a). The weight of females decreased by about 2.3 g from the pre-laying to early incubation phase, but a substantial part of this difference was caused by the presence of oviducal eggs in four of the six females collected and by the decrease in the weight of ovary and oviduct (1.45 g).

The lean dry weight of breast muscles remained constant during the phases concerned, while that of the body tended to have a peak value in the pre-laying phase. It decreased by about 80 mg (excluding the weight of ash, see Table 3) to the early phase of laying and then remained at an approximately constant level. The magnitude of the decrease would well suffice to cover the demands of one yolk. Because the decrease in the lean dry weight was small, it is suggested that in pied flycatchers the stores play only a minor rôle in protein metabolism during laying.

Pinowska (1979, fig. 8) noted that in the house sparrow the amount of proteins was greatest on the day of ovulation of the first egg, and decreased steadily towards the beginning of incubation. Jones & Ward (1976) showed that in the red-billed quelea the corresponding decrease was significant during the laying of the most common 3-egg clutch, the lowest reserves being observed just after completion of the clutch. Red-billed queleas invested about 150 mg protein in the three yolks, and the loss in the lean dry matter in their flight muscle amounted to about 160 mg for the period of laying (Jones & Ward 1976). The authors concluded that the flight muscle in this species was the main store for the yolk proteins. A suggestion that these stores represent special proteins required for egg production (Fogden & Fogden 1979) does not seem to hold good in the case of pied flycatcher since the dry weight of breast muscle and of the body remained stable from laying to early incubation.

Furthermore, Pulliainen & Peiponen (1981) point out the importance of availability of protein-rich seeds and accumulation of the reserves in initiating breeding activities in the redpoll, *Carduelis flammea.*

The dry weights of proteins in albumen and shell were 160 mg per egg in the pied flycather, these being invested during the last 24 hours prior to laying. In the domestic hen the albumen proteins are apparently transported to the oviduct after ovulation and so possibly no storage of egg-white proteins occurs (see review of Gilbert 1971). Also, the proteins secreted for the albumen and shell from the oviduct may originate in the pied flycatcher from food recently assimilated.

Obviously pied flycatcher females must rely mainly on foraging to meet the extra demands for proteins. All females collected had their stomachs partially full, or full, of insects. Thus, they had foraged during the early morning hours before they intended to lay their eggs. Courtship feeding may be another way to meet the nutrient demands, but only one author (Curio 1959) has reported that male pied flycatchers may occasionally feed the female even before laying. As the female is not very conspicuous during the early part of the breeding cycle, this habit may be more common than reported so far (see e.g. von Haartman 1958).

4.3. The rôle of fat reserves during laying

The rapid depletion of fat reserves from the peak value during laying in the red-billed quelea was interpreted by Jones & Ward (1976) as being correlated with maximal foraging for protein, while Fogden & Fogden (1979) suggested this reflected the loss of feeding time caused by searching for calcium-rich material for shell formation in the red-backed camaroptera, *Camaroptera brevicaudata*. In the house sparrow the lipid level of the body remained about the same during all the breeding phases (Pinowska 1979).

A steady loss in lipids was recorded here in the pied flycatchers from the pre-breeding to nesting phases. As one egg contains about 100 mg lipids, the loss in lipid reserves by 700 mg from pre-laying to early incubation would cover the demands of seven eggs. However, as suggested by Krapu (1974), the rôle of lipid reserves is not only to cover yolk demands. Its other important function is to safeguard the overall condition of birds. Thus, females with large depots are capable of foraging very selectively, i.e. of collecting special protein-rich food items or snail shells for calcium intake (Krapu 1974, Fogden & Fogden 1979). Heavier pied flycatcher females also lay larger numbers of eggs than lighter ones do (Askenmo 1982).

The situation in the pied flycatcher resembles that observed in many ducks. For instance, the pintail (Krapu 1974) and mallard, *Anas platyrhynchos* (Krapu 1979) enter the breeding season with large fat reserves, but rely mostly on foraging for the protein. According to Krapu (1974, 1979), the fat reserves are largely used to seek high quality food, rich in protein, and birds with large fat reserves may forage inefficiently, foraging not for calories but via invertebrates to secure the demands for the proteins.

4.4. Variation in ash-contents during laying

The decrease in the body ash-contents from the pre-laying to the incubation phase suggests that stored minerals, especially calcium, have been utilized in forming the eggs and eggshells. The calcium is known to be deposited as the medullary bone in birds (Simkiss 1961), and also small passerines like the house sparrow are known to deposite it. Other elements, such as zinc, may be stored in the bones of birds at laying time (Ojanen et al. 1975). When supplementing the calcium required for eggs, females eat snail shells or other calcium-rich material (Jones 1976, Schifferli 1977 and references therein). Fragments of snail shell were also recorded from the stomach of one pied flycatcher female out of the six collected during the laying phase.

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