J Comp Physiol B (1996) 166: 388–395

ORIGINAL PAPER

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Digestive tract function in the long-distance migratory garden warbler, Sylvia borin

Accepted: 20 May 1996

Abstract Digestive tract morphology and function of captive garden warblers (*Sylvia borin*) were measured during four stages of their endogenous circannual rhythm: before, during and after their autumn fattening prior to migration to wintering grounds in Africa, and after a partially simulated migratory flight. Food intake increased by 33% during fattening, utilization efficiency of dry matter tended to increase, and that of energy increased significantly ($P < 0.01$). This was because digestive tract capacity (measured as dry tissue mass) increased, so that mean retention time of food remained constant before, during and after fattening (80–84 min). After a 48-h period of starvation of fattened birds to partially simulate a migratory flight, food intake was lower on the first day of refeeding than on the next 4 days, and utilization efficiency was higher on that day, at least partly because of a longer mean retention time (111 min versus 78 min on the third day). Digestive tract dry tissue mass fell by 50% during starvation, and that of the small intestine by 63%. It is concluded that the garden warbler adapts to long-distance migration without feeding by rapidly reducing the size of its digestive tract, an expensive tissue to maintain, during migration in order to save weight and energy, and possibly also to supply part of the fuel and protein required for the flight. The cost of this strategy appears to be the time taken to rebuild the gut at stopover sites with food, but the low probability of finding such a site in the Sahara Desert means that this strategy is probably optimal for garden warblers.

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Introduction

The garden warbler, *Sylvia borin*, is a small (16–26 g) passerine bird that breeds in central and northern Europe but winters in eastern and southern Africa. The long autumn migratory flight is preceded by an intense period of hyperphagia and fattening (Bairlein 1985; Klaassen and Biebach 1994), but once the birds leave southern Europe they have little chance of finding food until they have crossed the Mediterranean Sea and Sahara Desert (Biebach 1990). The digestive tract of these birds therefore has to cope with two extremes in food intake; pre-migratory hyperphagia and migratory starvation. The small intestine of another small passerine, the American house wren, *Troglodytes aedon*, increased in length by 21% to accommodate a doubling in food intake in response to the energetic demands of subzero ambient temperatures (Dykstra and Karasov 1992), but the way in which the digestive tract of long-distance migratory passerines responds to the energetic demands of migration is unknown.

We used captive garden warblers fed a standard diet ad libitum and compared food intake, efficiency of dry matter and energy utilization, and gut morphometry before, during and after pre-migratory fattening. We also partially simulated a migratory flight by fasting one group of fattened birds for 48 h, then measured the same parameters of food intake and utilization, and gut morphometrics. Bairlein (1985) had previously described the endogenous annual pattern of food intake, body weight and nutrient utilization of captive garden warblers fed a similar standard diet but did not simulate migration, nor did he report on any gut parameters.

Materials and methods

Adult garden warblers were caught in Radolfzell, SW-Germany (approx. 48 °N, 9 °E) in August. They were maintained in two large

indoor aviaries until the following July, when they were divided into four groups of six birds stratified by moult stage and bm. They were transferred into individual cages 70 cm × 33 cm and 35 cm high and fitted with two perches, and were fed a standard diet of 30% hard boiled eggs, 25% commercial insect food (Eckrich), 21% curd, 15% bread crumbs, 6% ground eggshells and 3% beef heart (Gwinner et al. 1988) ad libitum. A single, large batch of the diet was prepared for the entire experiment and stored frozen in daily portions. All birds were weighed three times each week and, prior to experimental periods, any bird that lost body weight was given mealworms as a supplement to the standard diet. The temperature in the experimental room was maintained at 21 ± 2 °C throughout the study. Photoperiod was set initially at LD 16:8 but, from August 8, the light period was reduced by 30 min per week to mimic the shortening daylength experienced by wild birds over late summer and autumn in central Europe, in order to stimulate pre-migratory fattening. From September 19 photoperiod was set at LD 12.5:11.5 to mimic the more constant day length that migrating birds experience as they fly south in autumn.

Experimental design

The groups were randomly assigned to one of four treatments:
Group 1: Pre-fattening, late July, LD 16:8
Group 2: Fattening prior to migration, late August, LD 14:10
Group 3: Post-fattening, early October, LD 12.5:11.5
Group 4: "Simulated migration", mid October, LD 12.5:11.5

For Groups 1, 2 and 3 the experimental period consisted of an initial 5 days during which food intake and excretion were measured each day. Daily portions of the diet were transferred to a refrigerator the day before use and thus were thawed by the time of use. Samples of the daily portions of the diet were taken for immediate determination of dry matter and later determination of gross energy content. Fresh food equivalent to approximately 15 g dry matter was weighed into porcelain trays at 1000 hours each day, and fresh water was provided at the same time. Excreta were collected onto light aluminium trays made with a 3-mm lip. Trays were removed at 1000 hours, and immediately replaced by clean trays. Excreta were transferred quantitatively from the trays into tared 400-ml aluminium foil cups using a Perspex scraper and water from a wash bottle. Collected excreta were then evaporated to dryness at 80 °C over 72 h. Food residues, including spilled food on the aluminium trays, were collected at the time of feeding and transferred to similar tared cups, then dried at 80 °C for 24 h.

On the sixth day each bird was given a dose of 0.05 ml of a solution containing 15 mg Co-EDTA per os immediately after the collection period had been completed and fresh food provided. The dose was administered from a syringe through a soft plastic tube introduced down the oesophagus into the proventriculus. The aluminium collection trays were then checked at 30, 60, 90, 120, 150, 180, 240, 300 and 360 min after dosing, and any excreta present were collected and treated as above. These were later analysed for Co concentration and the results were used to calculate the MRT of the marker in the gut.

On the seventh day, 1–2 h after fresh food had been provided, all six birds in each group were weighed then sacrificed by decapitation. Trunk blood was collected into Eppendorf centrifuge tubes containing anticoagulant and immediately centrifuged to separate cells from plasma; the plasma was stored at − 60 °C for later analysis for melatonin. As soon as the trunk blood had been collected, the ventral surface of the bird was plucked, and a midline incision made to allow removal of the entire gastrointestinal tract, as well as the heart and liver. The tract was immediately cleared of adhering mesentery, pancreas and fat, weighed, then divided into proventriculus, gizzard, duodenum, remainder of small intestine (jejunum and ileum), and colon (from caecal "buds" to cloaca). The proventriculus was halved longitudinally; one half was weighed into a tared Eppendorf tube then placed on ice, and the other half was weighed into a tared 20-ml aluminium cup. The gizzard was opened and emptied of its contents then the whole organ was weighed into a tared aluminium cup. The duodenum and colon were emptied of their contents by gently squeezing them against the dissection board with a scalpel handle, then the whole tract segments were weighed into tared Eppendorf tubes and placed on ice. The jejunum plus ileum was similarly prepared, but was weighed into a tared aluminium cup. The heart was emptied of blood by squeezing, and the heart, liver and right and left pectoralis major (the main flight muscles) were also weighed into tared aluminium cups.

The three tissues weighed into the Eppendorf tubes (part of the proventriculus, the duodenum and colon) were stored at − 40 °C for later analysis for melatonin. The tissues weighed into aluminium cups (part of the proventriculus, gizzard, jejunum plus ileum, heart, liver and pectoralis major) were dried at 60 °C for 24 h to determine the dry mass of tissue. The dry matter content of the part-proventriculus was used to calculate the dry mass of proventriculus tissue used for melatonin assay, and that of the jejunum plus ileum was used to calculate the tissue dry mass of duodenum and colon used for melatonin assay; preliminary tests demonstrated that dry matter content of tissues was uniform along the length of the intestine. Procedures for the melatonin assays and findings from them will be reported in a separate paper.

For Group 4 ("Migration"), a migratory flight over desert was partially simulated by removing food (but not water) for 48 h. Food was then provided for the next 5 days to simulate conditions at a stopover site with food, and intake and efficiency of utilization of dry matter and energy were measured on each day of refeeding, following the same procedures as for Groups 1, 2 and 3 above. Mean retention time of digesta in the gut was measured on the first and third days of refeeding concurrently with the other measurements; experience with the other groups indicated that excreta collections as frequently as half-hourly during the day had no significant effect on food intake.

Following the 5-day collection period, group 4 birds were again deprived of food to partially simulate a further 48-h migratory flight over desert. All six birds were then sacrificed and tissues taken and measured following the same procedures as for groups 1, 2 and 3. Final body mass was 19.6 ± 2.9 g.

When ten wild-caught garden warblers (five first-year juveniles and five adults) from Radolfzell were sacrificed in August for other purposes, the same tissues as in the captive warblers were taken and analysed following the same procedures as for groups 1, 2, 3 and 4 above. This provided an opportunity to compare captive birds from group 1 (pre-fattening) with their wild counterparts. The wild birds were fed with mealworms, and were dissected two days after capture.

Chemical analyses

The gross energy content of food, food residues and excreta was determined in an automated adiabatic bomb calorimeter (Model C7000, IKA-Analysentechnik, Heitersheim, Germany). Samples were ground with a glass mortar and pestle, and subsamples of 0.5–1.0 g dry matter were used for determinations.

The concentration of cobalt in the excreta collected for measurement of mean retention time in the gut was determined by atomic absorption spectroscopy after digestion of organic matter with nitric acid. The dried excreta were first dissolved and washed down to the bottom of the aluminium cups with 2 ml nitric acid and digested at room temperature for 20 min. The cups were then placed in glass funnels standing in glass test tubes, and the bottom of the cup pierced with a glass rod. A further 8 ml nitric acid was used to wash all contents of the cup into the test tube using a syringe. Digestion was allowed to proceed at room temperature for a further 48 h. Deionized water (10 ml) was then added to the test tubes, the contents mixed, and the tubes centrifuged at 1000 *g* for 5 min to sediment undissolved inorganic materials. The clear supernatant

was then analysed for cobalt in an atomic absorption spectrophotometer (Model 939, Unicam, Kassel, Germany). Preliminary tests using repeated washings from the same cup showed that recovery of cobalt from excreta by this method was routinely greater than 95% with a single wash; this is because of the highly soluble nature of Co-EDTA. Nevertheless, a second wash was always used whenever any solids were left in the cup; the second washings were analysed separately and any cobalt detected was added to the first reading.

Data analysis

Efficiency of dry matter (energy) utilization used here is calculated as the difference between daily dry matter (energy) intake and daily excreta dry matter (energy) output expressed as a percentage of daily dry matter (energy) intake. This calculation is therefore *apparent* efficiency of utilization, because it does not account for endogenous loss of dry matter or energy. It is equivalent to apparent metabolizability (Robbins 1983), but *not* to apparent digestibility, nor to apparent assimilation efficiency, because it includes urinary as well as faecal losses of dry matter and energy.

Of several measures of food passage rate, the single most useful parameter is MRT, the average time that a digesta particle remains in the gut. It can be estimated as the mean time taken by an appropriate marker to be excreted following a pulse dose per os (Warner 1981). Co-EDTA satisfies most of the criteria for a solute marker in that it is inert, non-toxic, and not absorbed to any appreciable extent from the gut of most species (Udén et al 1980; Stevens and Hume 1995). Such a solute marker is appropriate because differential passage of solutes and particles is unlikely to be significant in these birds. MRT was calculated as

$$\frac{\sum_{i=1}^{n} M_i T_i}{\sum_{i=1}^{n} M_i}$$

where M_i is the amount of marker excreted at time T_i, and T_i is the midpoint between successive excreta collections.

All statistical tests were performed using SPSS/PC (1988). The significance of differences in dry matter and energy intake and excretion among groups 1, 2 and 3 was tested using ANOVA, and that of efficiency of utilization using ANCOVA with dry matter intake as the covariate. Differences in MRT among groups 1, 2 and 3 were tested for significance by ANOVA and between days 1 and 3 in group 4 by paired *t*-tests. Within group 4, differences in dry matter and energy intake, excretion and utilization efficiency between days were also tested by paired *t*-tests. The relationships between efficiency of utilization and intake of dry matter and energy were calculated by linear regression analysis.

Results

Performance of birds before, during and after fattening

The initial body masses of the birds when allocated to groups and treatments on July 11 were not significantly different. Group 1 birds (pre-fattening) virtually maintained mass during their 5-day collection period, and consumed 3.3 g dry matter per day, equivalent to 20% of body mass. In contrast, group 2 birds (fattening) ate 32% more dry matter and energy ($P < 0.001$), and increased in body mass by an average of 2.8 g in 5 days. Group 3 birds (post-fattening) ate similar amounts of dry matter to Group 1 birds, but were 33% heavier (Table 1).

The efficiency with which dry matter was used tended to be higher in group 2 birds than in the others ($P = 0.080$), and that of energy was significantly greater ($P < 0.01$) in the group 2 birds. Despite the large increase in food intake of group 2 birds, the MRT of the solute marker Co-EDTA did not differ among groups. Transit time (time of first appearance of marker after dosing), another useful parameter of digesta passage, could not be determined in this study because initial collections made at 30 min after dosing contained

Table 1 Performance of birds in groups: 1 (pre-fattening), 2 (fattening) and 3 (post-fattening). Data are means (± standard errors) of six birds per group over 5 days of collection. For body mass and mean retention times, data are means (± standard deviations) of six birds per group (*GE* gross energy, *ME* metabolizable energy). Daily intake = amount utilized

	Group		
	1	2	3
Body mass (g)			
Initial (July 11 – time of allocation to groups)	17.4 ± 1.3[a]	18.8 ± 0.8[a]	17.8 ± 1.5[a]
Mean (during collection period)	16.6 ± 0.6[a]	23.0 ± 0.5[b]	22.1 ± 0.9[b]
Change (per 5-day collection period)	+0.3 ± 0.1[a]	+2.8 ± 0.3[b]	+0.2 ± 0.2[a]
Dry matter (g)			
Daily intake	3.34 ± 0.23[x]	4.42 ± 0.20[y]	3.18 ± 0.36[x]
Daily excretion	1.37 ± 0.16[a]	1.61 ± 0.10[b]	1.28 ± 0.24[a]
Daily utilization	1.97 ± 0.25[x]	2.73 ± 0.16[y]	1.91 ± 0.13[x]
Utilization efficiency (%)	59.9 ± 2.2[a]	62.3 ± 1.5[a]	61.0 ± 2.3[a]
Energy (kJ)			
Daily intake (GE)	81.95 ± 5.19[x]	109.27 ± 8.47[y]	73.71 ± 7.58[x]
Daily excretion	22.17 ± 3.75[a]	22.94 ± 1.49[a]	19.04 ± 5.38[a]
Daily intake (ME)	59.78 ± 2.46[x]	86.43 ± 8.29[y]	54.67 ± 3.02[x]
Utilization efficiency (%)	73.9 ± 2.8[a]	78.4 ± 1.6[b]	75.8 ± 3.6[a]
Digesta passage			
Mean retention time (min) of Co-EDTA	83.2 ± 20.2[a]	84.2 ± 12.5[a]	79.7 ± 21.7[a]

[a, b] Means on the same line bearing different superscripts differ at $P < 0.01$
[x, y] Means on the same line bearing different superscripts differ at $P < 0.001$

Table 2 Performance of birds in group 4 during refeeding following a 48-h fast. Initial body mass (July 11–time of allocation to groups) was 18.7 ± 2.3 g, and before fasting was 24.6 ± 3.0 g. Data are means (± standard deviation) of six birds (*GE* gross energy, *ME* metabolizable energy). Daily intake = amount utilized; nm = not measured

	Day of refeeding				
	1	2	3	4	5
Body mass (g)	20.8 ± 2.9	22.0 ± 2.7	22.6 ± 2.7	23.1 ± 2.8	23.2 ± 2.8
Body mass change (g per day)	+1.2 ± 0.6	+0.6 ± 0.1	+0.5 ± 0.3	+0.1 ± 0.1	+0.4 ± 0.3
Dry matter (g)					
Intake	2.99 ± 0.52^{a}	3.51 ± 0.46^{b}	3.42 ± 0.63^{ab}	3.19 ± 0.58^{ab}	3.27 ± 0.38^{ab}
Excretion	1.02 ± 0.20^{a}	1.38 ± 0.28^{b}	1.36 ± 0.39^{b}	1.28 ± 0.41^{b}	1.32 ± 0.16^{b}
Utilization	1.97 ± 0.34^{a}	2.13 ± 0.25^{a}	2.07 ± 0.38^{a}	1.91 ± 0.29^{a}	1.95 ± 0.35^{a}
Utilization efficiency (%)	65.8 ± 2.5^{a}	60.9 ± 4.4^{b}	60.7 ± 5.7^{b}	60.5 ± 6.6^{b}	59.4 ± 5.8^{b}
Energy (kJ)					
Daily intake (GE)	76.42 ± 11.57^{a}	91.56 ± 12.24^{b}	85.89 ± 13.38^{ab}	82.43 ± 13.72^{ab}	85.45 ± 12.05^{ab}
Daily excretion	12.69 ± 2.11^{a}	20.54 ± 5.86^{b}	20.32 ± 11.65^{b}	18.22 ± 10.81^{b}	17.69 ± 4.46^{b}
Daily intake (ME)	63.73 ± 9.75^{a}	71.03 ± 8.17^{a}	65.57 ± 9.01^{a}	64.21 ± 9.49^{a}	67.76 ± 11.86^{a}
Utilization efficiency (%)	83.4 ± 1.3^{x}	77.8 ± 3.6^{y}	77.1 ± 9.4^{y}	78.7 ± 9.4^{y}	79.1 ± 5.3^{y}
Digesta passage					
Mean retention time (min) of Co-EDTA	110.8 ± 12.0^{x}	nm	78.0 ± 15.5^{y}	nm	nm

^{a,b} Means on the same line bearing different superscripts differ at $P < 0.01$
^{x,y} Means on the same line bearing different superscripts differ at $P < 0.001$

marker in all but one instance; it was not possible to collect excreta at more frequent intervals without excessive disturbance to the birds.

Performance of birds during refeeding after simulated migration

Body mass fell by 15% during the 48-h fast designed to partially simulate a migratory flight over desert (Table 2). When food was made available after this period, dry matter intake did not immediately increase maximally, so that intake on the first day of refeeding was lower ($P < 0.05$) than on the second day; there were no significant differences in food intake after this day. The low food intake on the first day of refeeding coincided with higher ($P < 0.05$) efficiencies of utilization of both dry matter and energy on that day compared with the other days, and also with a longer ($P < 0.01$) MRT (111 versus 78 min) on the third day.

When efficiency of utilization of dry matter was plotted against dry matter intake, the data from groups 1, 3 and 4 were significantly and linearly related with negative slope (Fig. 1). In contrast, data from group 2 (fattening) birds formed a separate population; all points were outside one standard deviation of residuals from the regression line. Results for efficiency of energy utilization formed a similar pattern (Fig. 2).

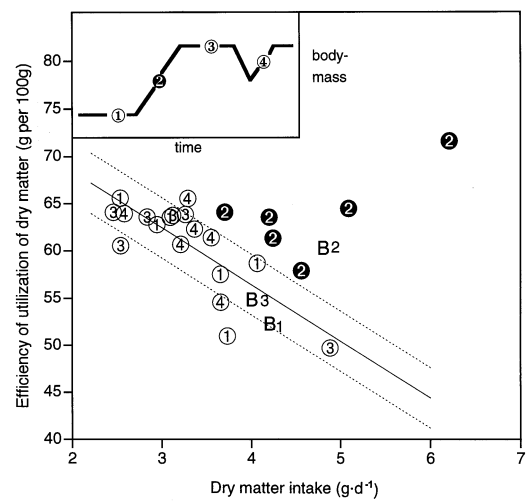

Fig. 1 Relationship between efficiency of utilization of dry matter and dry matter intake of garden warblers from groups 1 (prefattening), 3 (post-fattening) and 4 (fattened birds after 48 h starvation) (see insert for schematic representation of the four experimental groups). *Solid line* is linear regression [$Y = 80.38 = 6.01\ X$; multiple $R = 0.779$ ($P < 0.001$)]. *Broken lines* are ± one SD (2.96) of residuals from the regression line. All group 2 (fattening) birds lie outside the broken lines. *B1, B2* and *B3* are mean values for corresponding phases from Bairlein (1985) for comparison.

Responses of the gut and other tissues

There were no significant differences in the fresh weight of the gastrointestinal tract, either with or without contents, between wild-caught birds and our group 1 birds, or between group 1 and groups 2 and 3 (Table 3). However, group 4 was significantly lower ($P < 0.01$) than the other groups on both bases. Similarly, the dry matter percent of tissues did not differ between wild-caught and group 1 birds. However, tissue dry matter percent of the gizzard, intestine, liver, heart and pectoralis major significantly increased from group 1 to

group 2 or group 3. For the gizzard and intestine, tissue dry matter percent of birds from group 4 was markedly ($P < 0.001$) lower than group 3.

Among the wild-caught birds, tissue dry masses were generally similar for juveniles and adults, except that the total gastrointestinal tract was heavier in the juveniles ($P < 0.05$), and the heart lighter ($P < 0.05$) (Table 4). The gizzard of the wild birds was heavier than that of group 1 birds ($P < 0.001$) (Fig. 3). The heart of the adult wild birds (but not the juveniles) was heavier than group 1 birds ($P < 0.05$).

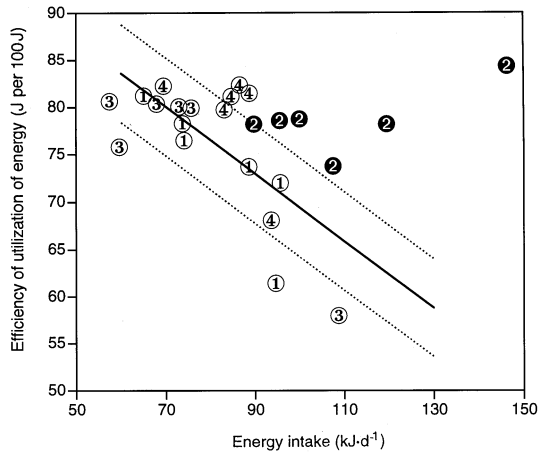

Fig. 2 Relationship between efficiency of utilization of energy and gross energy intake of garden warblers from groups 1 (pre-fattening), 3 (post-fattening) and 4 (fattened birds after 48 h starvation) (see insert in Fig. 1 for schematic representation of the four experimental groups). *Solid line* is linear regression [$Y = 104.80 - 0.36\,X$; multiple $R = 0.686$ ($P < 0.01$)]. *Broken lines* are $\pm$ one SD (5.21) of residuals from the regression line. All group 2 (fattening) birds lie on or outside the broken lines.

As with tissue dry matter percent, tissue dry mass of the gizzard, heart and flight muscles increased significantly from group 1 to group 2 or group 3. The small intestine tended to increase from group 1 to group 3. Group 4 birds had a significantly lighter gizzard by 30% ($P < 0.01$), small intestine by 63% ($P < 0.01$), total gut by 50% ($P < 0.001$) and liver by 24% ($P < 0.01$) than group 3, but the heart and flight muscles were maintained (Fig. 3).

Discussion

Results from this study are conveniently discussed in three parts: wild-caught versus captive birds before fattening (group 1); responses of the gut and other tissues to pre-migratory fattening (groups 1, 2 and 3); and the effects of a partially simulated migratory flight (group 4).

Wild versus captive birds before fattening

The wild birds caught at Radolfzell were at a similar moult stage to the birds in group 1 at the time of their collection period. Most parameters measured were similar between the two groups. The wild birds differed from the group 1 birds only in their larger gizzard, probably reflecting a lower quality natural diet than the captive diet used in this study and, in the case of the adults, their larger heart, a difference which may be related to the migratory flights of the adult wild birds during the period of captivity of the experimental birds. Among the wild birds, the heavier gastrointestinal tract of the juveniles probably reflects the greater energy requirements for growth compared with maintenance; their smaller heart probably reflects the lack of a migratory flight.

Table 3 Body mass and gastrointestinal tract mass and dry matter percent of groups 1,2,3 and 4 birds compared with wild-caught juvenile (first year) and adult garden warblers. Data are means ($\pm$ standard deviations). Group 4 birds after second 48-h starvation period

	Wild-caught		Captive group			
	Juveniles ($n = 5$)	Adults ($n = 5$)	1 ($n = 6$)	2 ($n = 6$)	3 ($n = 6$)	4 ($n = 6$)
Body mass (g)	—	—	17.4 ± 1.3	18.8 ± 0.8	17.8 ± 1.5	18.7 ± 2.3
Initial (July 11)	16.5 ± 0.5	18.0 ± 1.4	17.4 ± 1.1	23.8 ± 1.1	22.6 ± 2.7	19.6 ± 2.9
Final	(Aug 31)	(Aug 31)	(Aug 7)	(Sept 3)	(Oct 10)	(Oct 13)
Gastrointestinal tract (g)						
Fresh mass, with contents	1.79 ± 0.05^{a}	1.65 ± 0.28^{a}	1.69 ± 0.22^{a}	1.66 ± 0.24^{a}	1.82 ± 0.30^{a}	1.02 ± 0.06^{b}
Fresh mass, less contents	1.21 ± 0.16^{a}	1.00 ± 0.12^{a}	1.11 ± 0.22^{a}	1.05 ± 0.11^{a}	1.24 ± 0.28^{a}	0.69 ± 0.07^{b}
Tissue dry matter (%)						
Proventriculus	24.1 ± 1.1^{a}	24.7 ± 1.5^{a}	24.9 ± 1.7^{a}	27.7 ± 3.4^{a}	23.4 ± 4.1^{a}	25.5 ± 1.2^{a}
Gizzard	32.0 ± 0.6^{ax}	33.5 ± 2.4^{ax}	32.3 ± 1.5^{ax}	34.9 ± 1.3^{bx}	39.2 ± 3.2^{cy}	33.9 ± 3.0^{ax}
Intestine (including colon)	31.4 ± 2.1^{ax}	31.2 ± 5.6^{ax}	30.7 ± 3.1^{ax}	40.3 ± 1.7^{by}	41.2 ± 3.2^{by}	33.2 ± 1.3^{ax}
Liver	30.8 ± 0.6^{ax}	31.0 ± 2.2^{ax}	31.0 ± 1.2^{ax}	34.7 ± 1.0^{by}	34.9 ± 0.4^{by}	35.9 ± 1.9^{by}
Heart	25.1 ± 0.4^{ax}	26.4 ± 0.4^{bx}	25.1 ± 1.0^{abx}	27.6 ± 0.4^{cy}	28.2 ± 0.7^{cy}	28.5 ± 1.6^{cy}
Pectoralis major	29.4 ± 0.7^{ax}	29.9 ± 0.8^{ax}	28.6 ± 0.9^{ax}	31.6 ± 1.3^{by}	31.6 ± 1.4^{by}	30.9 ± 1.1^{by}

[a, b] Means on the same line bearing different superscripts differ at $P < 0.01$
[x, y] Means on the same line bearing different superscripts differ at $P < 0.001$

Table 4 Dry tissue mass (g) of the gut and other tissues of wild-caught garden warblers. Data are means (± standard deviations); ns = not significant

	Juveniles ($n = 5$)	Adults ($n = 5$)	Statistical difference (P)
Proventriculus	14.6 ± 1.8	12.6 ± 1.1	ns
Gizzard	126.4 ± 13.5	117.2 ± 21.6	ns
Small intestine	212.2 ± 23.4	173.4 ± 49.1	ns
Colon	21.0 ± 11.6	14.6 ± 2.5	ns
Total gut	374.2 ± 34.5	317.6 ± 40.4	< 0.05
Liver	185.8 ± 27.2	180.2 ± 35.1	ns
Heart	45.4 ± 3.0	58.8 ± 9.3	< 0.05
Pectoralis major	628.6 ± 40.5	671.8 ± 75.0	ns

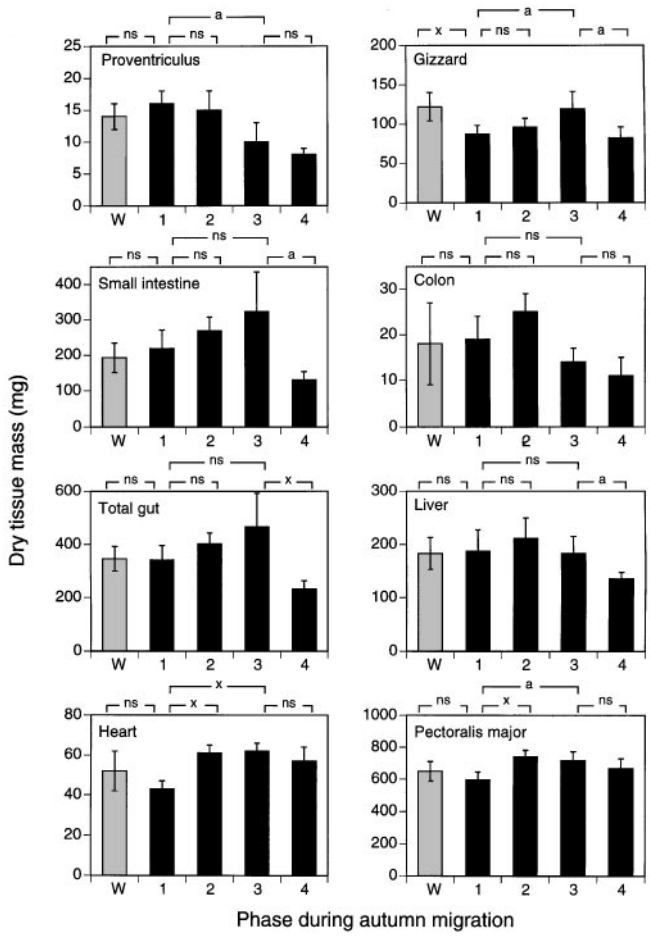

Fig. 3 Dry tissue mass (mean ± SD) of the gut and three other tissues of garden warblers during four phases of the autumn migration (see insert in Fig. 1 for schematic representation of the four experimental groups). Wild-caught birds (five juveniles plus five adults) (W) also shown for comparison with group 1 captive birds. Statistical comparisons (one-way ANOVA): *ns* = not significant; *a* = significant at $P \leq 0.01$; *x* = significant at $P \leq 0.001$.

Responses to pre-migratory fattening

The remarkable and rapid increase in body mass of our garden warblers over a short period in autumn is a well-described phenomenon (King 1961; King and Farner 1965; Berthold 1975). The endogenous circannual rhythmicity in migratory events, including pre-migratory fattening (Gwinner 1986), was well illustrated by Bairlein (1985) when he held captive garden warblers under conditions of constant photoperiod (LD 12:12) and temperature and fed a standard diet similar to the one used here. The pattern of body mass change in our birds closely matched that of Bairlein's over the same period of the year.

The 33% increase in dry matter intake during fattening exceeded the 14% recorded by Bairlein (1985), as also did the rate of body mass gain over the 5-day collection period (2.8 versus 1.5 g). The increased food intake was accompanied by a significant improvement in efficiency of utilization of energy, as Bairlein (1985) found for dry matter. This response can only be explained if gut capacity increased concurrently; otherwise increased food intake would be expected to result in shorter retention times in the digestive tract and less complete digestion (Stevens and Hume 1995). However, mean retention time in the gut was constant (Table 1), and gastrointestinal tract tissue dry mass tended to increase during fattening (group 2). Together these data demonstrate that, during pre-migratory fattening in garden warblers, the digestive tract expands in response to greater food intake, with no loss in digestive efficiency. In fact the response is such that digestive efficiency actually increases, at least for energy, as Bairlein (1985) found for dry matter.

Figures 1 and 2 clearly demonstrate this phenomenon; all points from the fattening birds fall outside one standard deviation of residuals from the regression line describing the relationship between utilization efficiency and food intake from the other three collection periods. The means of the equivalent first three periods from Bairlein (1985) are included in Fig. 1 for comparison. The similarity in response to fattening in the two studies is readily apparent. Bairlein's pre- and post-fattening birds ate more dry matter than ours in order to maintain body mass, indicating that their diet, although similar to ours, was not of as high a quality. Nevertheless, the means fall close to our regression line, indicating similar gut dimensions. Bairlein's fattening birds responded in the same manner as ours, as indicated by the close match with our fattening birds, and therefore must also have increased their gut capacity. Thus the present study provides an explanation for the increases in efficiency reported by Bairlein (1985; 1990), and corrects the statement by Klaassen and Biebach (1994) that garden warblers apparently do not have special morphological or physiological adaptations for pre-migratory hyperphagia.

In addition to the trend for gastrointestinal tissue mass to increase during fattening, there were significant increases in the tissue mass of the heart and flight muscles, which can be interpreted as further preparations for migration. The trend for increases in the mass

of the gastrointestinal tract continued into the post-fattening period, particularly for the gizzard and small intestine. Increases in the tissue dry matter percent of the gizzard, intestine, liver, heart and flight muscles in group 2 birds are indicative of increased fat content because, unlike protein and carbohydrate, fat is stored without accompanying water. Shah et al. (1978) demonstrated that enzyme and hormone activities in the liver, the site of lipogenesis, increased during premigratory fattening in starlings (*Sturnus roseus*) and white wagtails (*Motacilla alba*). The dry matter (and thus probably fat) content of the gizzard tissues continued to increase in group 3 (post-fattening) birds.

Effects of a partially simulated migratory flight

By depriving the fattened birds of access to food for 48 h we simulated a natural event. When migrating warblers leave southern Europe they may be without food for up to 3 days (Moreau 1961). In the captive situation we were not able to simulate actual flight, and thus the energetic demands of migration. However, it is well known that when captive fattened garden warblers are deprived of food they exhibit increased night-time activity – migratory restlessness or "Zugunruhe" (Biebach 1985; Gwinner et al. 1985). Thus, there is at least some, albeit modest, increase in energy expenditure; Klaassen and Biebach (1994) calculated that the activity cost of Zugunruhe in starved pre-migratory garden warblers was equivalent to 16.5 $kJ \cdot day^{-1}$ over and above their basal metabolic rate of about 22 $kJ \cdot day^{-1}$.

The most striking effect of 48 h starvation was the 50% loss in the tissue dry mass of the gastrointestinal tract, and the even bigger loss (63%) of the small intestine (Fig. 3). This can be interpreted as the loss of a tissue that is expensive to maintain (Stevens and Hume 1995) and, when not in use, can be used as a source of both metabolic energy to help power the migratory flight, and perhaps of amino acids to replace those lost from the flight muscles through normal protein turnover. The cost of this "strategy" is the time and resources required to rebuild the gut when food becomes available again.

Thus, food intake was depressed on the first day of refeeding compared with the average intake of group 3 birds and with subsequent days of the refeeding period for group 4 birds. The low food intake, together with the significantly slower passage rate of food through the gut (MRT 42% longer than on day 3), are sufficient to explain the higher utilization efficiency seen on day 1 (Table 2). However, it must be remembered that the efficiency data are apparent efficiencies, and the possibility remains that lower endogenous losses in both faeces and urine (Guglielmo and Karasov 1993) on the first day may have contributed to the higher efficiency of utilization of both dry matter and energy. The other possibility, that some food was still in the gut at the end of day 1, is remote as garden warblers feed only in the light phase, and the MRT was less than 2 h even after 48 h without food; the amount of food still in the gut 11.5 h after cessation of feeding would thus be expected to be negligible.

The finding that food intake increased to a maximum of 71 kJ ME on the second day of refeeding suggests that rebuilding of the digestive system in our birds may have been fuelled mainly from stored fat; average body mass after 48 h of starvation was 20.8 g, still 2.1 g above their initial body mass. This contrasts with the study of Klaassen and Biebach (1994) in which garden warblers were starved for periods of up to 6 days in order to reduce body mass to a common level of 17 g, when fat reserves should be almost exhausted (Biebach et al 1986); consequently, food intake continued to increase from a low of less than 40 kJ ME per day to a maximum of more than 100 $kJ \cdot day^{-1}$ over 5–10 days. In this case rebuilding of the gut may have been fuelled mainly from food over a longer period. This appears to be the case with many migratory species; they initially only maintain or even lose body mass at a stopover site, then show only moderate increases in body mass despite abundant food availability (Nisbet et al. 1963; Langslow 1976; Rappole and Warner 1976; Biebach et al. 1986; Moore and Kerlinger 1987). However, other explanations are plausible and none is mutually exclusive. These include time to establish a feeding territory (Rappole and Warner 1976) and handling shock during repeated captures (Muller and Berger 1966).

We have no data on the rate of rebuilding of gut tissue mass in our study, but it is known from the work of Secor et al. (1994) and Secor and Diamond (1995) that increases in gut tissue mass when previously fasted snakes are fed are greatly exceeded by increases in small intestinal transporter activities, with the result that increases in function are up to 20 times increases in gut tissue mass. Thus small changes in gut tissue dry mass can represent very large changes in gut hydrolytic and absorptive function. The tissue dry mass of the small intestine of group 4 birds was only 40% of that of group 3 birds. Part of this difference is probably due to fat mobilization during starvation, as suggested by the decrease in percentage dry matter of the intestinal wall from 41 to 33% over the 48-h period without food (Table 3). Klaassen and Biebach (1994) calculated that 73% of whole-body tissue catabolized during starvation and deposited during refeeding consisted of fat. However, protein loss from the intestinal wall must also occur to account for the reduction in gut function (e.g. capacity, motility, hydrolytic activity) that is reflected in the birds by lower food intakes on the first day of refeeding. Further, more detailed study is required to ascertain the relative contributions of fat and protein to changes in gut

tissue mass during migratory flight and refeeding at stopover sites where food is available.

In contrast to the intestine, tissue dry mass of the heart and flight muscles was maintained during the starvation period (Fig. 3). Conservation of these tissues is essential in these long-distance migrants because the chance of finding food at a stopover site is extremely small, and further flight after resting on the ground during daylight hours is an almost certain requirement for survival.

Acknowledgements We thank Anke Dotterweich, Monika Trappschuh and Ingrid Schwabl for skilled technical assistance and Professor E Kienzle and Antje Wetzel for the cobalt analyses. The study was carried out while the senior author held a Research Fellowship from the Alexander von Humboldt Foundation.

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Communicated by G. Heldmaier