

Effect of food restriction on the energy metabolism of the Chinese bulbul (*Pycnonotus sinensis*)

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ABSTRACT

Food resources play an important role in the regulation of animals' physiology and behavior. We investigated the effect of short-term food restriction on metabolic thermogenesis of Chinese bulbuls (*Pycnonotus sinensis*) by measuring changes in body mass, body fat, basic metabolic rate (BMR), and organ mass of wild-caught Chinese bulbuls from Wenzhou, China. Short-term food restriction induced a significant decrease in body mass and body fat but body mass returned to normal levels soon after food was no longer restricted. Food restriction caused a significant reduction in BMR after 7 days ($P < 0.05$), which returned to normal levels after food restriction ceased. Log total BMR was positively correlated with log body mass ($r^2 = 0.126$, $P < 0.05$). The dry masses of livers and the digestive tract were higher in birds that had been subject to temporary food restriction than in control birds and those subject to continual food restriction ($P < 0.001$ and $P < 0.05$, respectively). There was also significant differences in the dry mass of the lungs ($P < 0.05$), heart ($P < 0.01$), and spleen ($P < 0.05$) in birds subject to short-term food restriction compared to control birds and those subject to continual food restriction. BMR was positively correlated with body and organ (heart, kidney and stomach) mass. These results suggest that the Chinese bulbul adjusts to restricted food availability by utilizing its energy reserves, lowering its BMR and changing the weight of various internal organs so as to balance total energy requirements. These may all be survival strategies that allow birds to cope with unpredictable variation in food abundance.

Keywords: Basic metabolic rate; Energy metabolism; Food restriction; *Pycnonotus sinensis*

INTRODUCTION

Migrating birds alternate between periods of short-term food

restriction during flight and periods of increased food intake at stopover sites (Bairlein, 1987; Pierce & McWilliams, 2004). The capacity to sustain a period of food restriction can be affected by external factors such as food intake and ambient temperature (Kendeigh, 1945; Ni et al, 2011), or internal factors such as body mass, body composition (Blem, 2000; Liknes & Swanson, 2011a) and the amount of stored fat (Burns, 2013; Liknes et al, 2014; Sartori et al, 1995).¹

Shallow nocturnal hypothermia, hypometabolism during periods of restricted food abundance, or fasting that induces adaptive changes in metabolism, are wide-spread facultative mechanisms for reducing energy requirements in birds (McKechnie & Lovegrove, 2002; Reinertsen, 1996), e.g., young Japanese quails (*Coturnix japonica*) (Schew & Ricklefs, 1998; Wall & Cockrem, 2009) respond by either actively decreasing their body temperature by 2 °C or reducing heat production by approximately 40% during fasting in thermoneutrality. Adaptive and active decreases in energy expenditure in response to food shortage can be pronounced, e.g., some galliformes can reduce their metabolic rate by 30-40% in thermoneutral conditions eventually entering torpor (Prinzinger & Siedle, 1988; Schew & Ricklefs, 1998). Such changes to heat production include changes in both physiology and metabolism (Marjoniemi, 2000). Theoretically, basal metabolic rate (BMR) is the minimum metabolic rate required for maintenance in endotherms (Swanson, 2001, 2010). It often serves as a baseline for comparisons of the metabolic costs of activities within species, and for comparisons of the "cost of living" among species or species groups (Kersten & Piersma, 1987; McNab, 1988; McKechnie & Wolf, 2004; Wiersma et al, 2007). BMR has become an important parameter for interspecific and intraspecific comparisons of energy metabolism that can indicate the relative energy consumption of different individuals

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and species, and thereby infer animals' degree of adaptation to various environment models (Liknes et al, 1996; McNab, 1997).

For homeotherm, the ability to regulate BMR, energy reserves, morphology, and organ function can be an effective way of making short-term adaptations to unpredictable, or reduced, food resources (Gebhardt-Henrich & Richner, 1998; Liknes & Swanson, 2011b; Vézina & Williams, 2003). Recent studies have shown that some bird species migrate with relatively small digestive organs and a lower digestive capacity (Bauchinger, 2002; Chediack et al, 2012; McCue, 2010; Pierce & McWilliams, 2004). One hypothetical explanation is that atrophy of these organs reduces BMR and wing loading thereby increasing flight capacity (Karasov et al, 2004). The 'digestive adaptation paradigm' (Karasov et al, 2004) suggests that, either decreasing digestive capacity, and/or down-regulating digestive tract activity to save energy, compensates for food shortage during migration by more efficient energy processing.

The Chinese bulbul (*Pycnonotus sinensis*) is a small passerine that breeds throughout central, southern and eastern China (MacKinnon & Phillipps, 2000; Zheng & Zhang, 2002). Within its natural range, the Chinese bulbul preferentially inhabits scrubland, open woodland, secondary forest, parks, gardens and villages on plains and hills up to 1 000 m a. s. l. (Zheng & Zhang, 2002). Chinese bulbuls are omnivorous, feeding primarily on arthropods (insects and spiders) and gastropods (snails and slugs) in the breeding season, but also eating fruits and seeds in autumn and winter (Peng et al, 2008; Zheng & Zhang, 2002). Chinese bulbul have been found to have higher temperature, lower BMR and a relatively wide thermal neutral zone (TNZ) (Zhang et al, 2006), as well as an obvious circadian rhythm and seasonal variations in the metabolic heat production (Zheng et al, 2008a; Zhou et al, 2010). We here test the following hypotheses: (1) during the migration, Chinese bulbul response to food shortage by the change of plasticity; and (2) the response to food deprivation is flexibility of body composition. We predict that Chinese bulbuls will show an increase in digestive efficiency and/or decreases in body mass, fat reserves and BMR during food restriction; their digestive tract mass will be adjusted in response to food availability; and the birds will regain lost condition after the period of food restriction ends.

MATERIALS AND METHODS

Animals

Chinese bulbuls were captured using mist nets in Wenzhou city, Zhejiang Province, China in May 2011. The climate is warm-temperate, with an average annual rainfall of 1 300 mm spread across all months with slightly more precipitation during spring and summer. Mean daily temperature is 18 °C. Body mass (to the nearest 0.1 g) was determined immediately upon capture with a Sartorius balance (model BT25S). Birds were transported to the laboratory on the day of capture, housed individually in metal cages (length×width×height, 50 cm×30 cm×20 cm) with a perch and containers for water and food and maintained at 21±1 °C with a 12L:12D cycle (lights on at 0600h). Food (20% crude protein, 6% crude fat, 4% crude fiber, 1%

Calcium, 0.5% Lys, 0.5% Met+Cys, Jiangsu Xie Tong Biological Engineering Company Ltd.) and water were supplied *ad libitum*. Birds were acclimated to these conditions for at least 2 weeks before experiments began. All animal procedures were licensed under the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences.

Experiment 1

In order to examine the effects of food limitation on the body mass and digestive efficiency of Chinese bulbuls, we randomly assigned 16 bulbuls to either a control ($n=8$) or a treatment group ($n=8$). Birds in the control group continued to be provided with food *ad libitum* whereas those in the treatment group were provided with 33.3% of the usual quantity of food for 7 days after which food was once again provided *ad libitum* for 8 days (Karasov et al, 2004).

Body mass and food intake were measured throughout the experiment on a daily basis; body mass was measured daily between 2000h and 2200h. Energy intake and digestibility were measured by collecting, manually separating and oven-drying (60 °C) food residues and feces to obtain their dry mass, after which their caloric content was determined with a C200 oxygen bomb calorimeter (IKA Instrument, Germany). Gross energy intake (GEI) ($Q_{food}=14\ 374$ kJ/g), feces energy (FE), digestible energy intake (DEI), and digestibility of energy were calculated according to Grodzinski & Wunder (1975) and Ni et al (2011):

$$\text{GEI (kJ/day)} = \text{dry food intake (g/day)} \times \text{caloric value of dry food (kJ/g)} \quad (1)$$

$$\text{FE (kJ/day)} = \text{dry mass of feces (g/day)} \times \text{caloric value of dry feces (kJ/g)} \quad (2)$$

$$\text{DEI (kJ/day)} = \text{GEI (kJ/day)} - \text{FE (kJ/day)} \quad (3)$$

$$\text{Digestibility (\%)} = \text{DEI (kJ/day)} / \text{GEI (kJ/day)} \times 100\% \quad (4)$$

Experiment 2

To examine the effects of different durations of food limitation on BMR, organ mass and body fat over time, 48 bulbuls were randomly assigned to six groups ($n=8$ in each group). These were then assigned to the following experimental groups: (1) a control group in which birds were supplied with food and water *ad libitum*; (2) four continual food-restriction (CFR) groups in which birds were provided with 33.3% of the usual amount of food for different periods of time (i.e. 1 day, 3, 5, and 7 days); (3) a temporary food-restriction (TFR) group in which birds were provided with 33.3% of the usual amount of food for 7 days after which they were fed *ad libitum* for 8 days.

Metabolic trials

Metabolic rates of bulbuls were measured with an open-circuit respirometry system (AEI Technologies Model S-3A/I, USA). For these measurements, birds were first acclimated in darkness for 1 h inside 3.5 L plastic metabolic chambers housed inside a temperature-controlled cabinet capable of regulating temperature within ±0.5 °C of a defined setpoint (Artificial climatic engine BIC-300, China). Air was scrubbed of water and CO₂ before entering, and after exiting the metabolic

chamber with Drierite and Ascarite. Dry CO₂-free air was pumped through the chamber at 300 mL/min using a flow control system (AEI technologies R-1, USA) calibrated with a general purpose thermal mass flowmeter (TSI 4100 Series, USA) (McNab, 2006). The fractional concentration of O₂ in inlet chamber air was determined using an oxygen sensor (AEI Technologies N-22M, USA). Oxygen consumption rates were measured at 30±0.5 °C, which is within the thermal neutral zone of Chinese bulbuls (Zheng et al, 2008a). Oxygen content of excurrent air was recorded at 10 s intervals for 1 h, after the 1 h equilibration period. BMR was calculated for each individual as the average of the 30 lowest consecutive oxygen consumption recordings (about 5 min). Food was removed 4 h before each test to provide post absorptive conditions. Metabolic rates of bulbuls were calculated from equation 2 of Hill (1972), and expressed as O₂ (mL)/g/h corrected to standard temperature and pressure, dry (STPD) conditions (Schmidt-Nielsen 1997). All measurements were made daily between 2000h and 2400h.

Measurement of organ mass

Birds were euthanized at the end of the experiment and their brain, heart, lungs, liver, kidneys, gizzard, small intestine, rectum and pectoral muscle were removed and weighed to the nearest 0.1 mg. Internal organs were dried to constant mass over 2 d at 60 °C and weighed to 0.1 mg (Liu & Li 2006; Williams & Tieleman, 2000; Zheng et al, 2008a).

Carcasses were dried to constant mass in an oven at 60 °C, and then weighed (to 1 mg) to determine their dry mass. Total fat was extracted from the dried carcasses by ether extraction in a Soxtec 2050 Soxhlet apparatus (FOSS Instrument, Germany). Body fat content was calculated according to Dawson et al (1983) and Zhao et al (2010):

$$\text{Body fat content (\%)} = \frac{\text{total fat of carcass}}{\text{wet carcass mass (mg)}} \times 100\% \quad (5)$$

Statistical analyses

We used SPSS (version 12.0 for Windows) for all statistical analyses and considered $P < 0.05$ as significant for all statistical tests. All results are expressed as means ± SE. Distributions of all variables were tested for normality using the Kolmogorov-Smirnov test. Non-normally distributed data were log-transformed. Repeated-measures analysis of variance (RM-ANOVA) was used to determine the significance of changes in body mass. Differences among groups were determined by one-way ANOVA. The significance of differences in BMR and organ mass were determined using ANCOVA with M_b as the covariate. Fisher's least significant difference (LSD) *post hoc* test was used to detect which groups differed significantly. We used least-squares linear regression to test for allometric correlations between log dry organ mass and log BMR with log M_b . If allometric correlations for organ masses were significant, we calculated residuals from the allometric equations and regressed the residuals of log dry organ mass against those of log BMR to determine if the mass of specific organs was significantly correlated with BMR. If allometric correlations were not significant, we regressed raw values for log dry organ mass

against log BMR to test for BMR-organ mass correlations (Zheng et al, 2013).

RESULTS

Variation in body mass

There was no significant difference in the initial body mass of the Chinese bulbuls assigned to the control, the four CFR, or the TFR, experimental groups (ANOVA, $F_{(5,30)} = 0.137$, $P = 0.982$, Figure 1, Figure 3A) but after acclimation a significant difference in body mass between treatment and control groups was apparent (RM ANOVA, $F_{(1,3)} = 532.8$, $P < 0.01$; LSD test, 10 day, $P > 0.05$; other groups, $P < 0.01$, Figure 1). There was a significant, negative, linear relationship between body mass and the duration of food restriction in the TFR group ($r^2 = -0.925$, $P < 0.01$, Figure 1) but the body mass of the TFR group increased to become essentially the same as that of the control 2 days after the end of food restriction ($P > 0.05$, Figure 1).

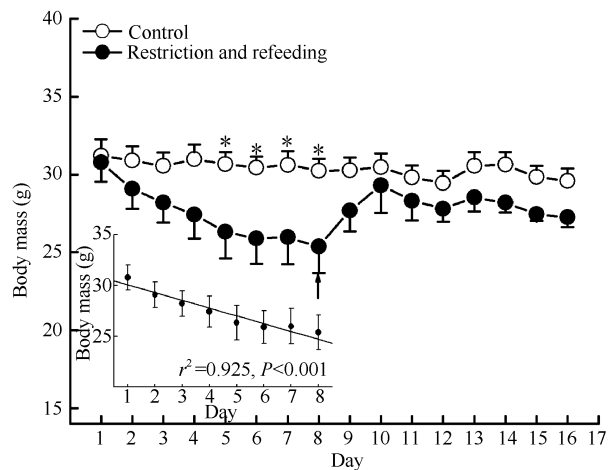


Figure 1 Relationship between body mass and food availability in the Chinese Bulbul (*Pycnonotus sinensis*)

Arrow indicates end of a 7 day period of temporary food restriction (TFR); * : $P < 0.05$.

Digestibility and body fat

Digestibility of acclimatized control birds was 35.4%, 6.6%, 17.7% and 22.4% lower than that of the 1 day, 3 day, 5 day and 7 day acclimatized CFR groups, respectively. Digestibility of the 1 day and 7 day CFR groups was significantly higher than that of both the control and TFR groups (LSD test, $P < 0.01$, Figure 2). Digestibility of the TFR group was essentially the same as that of the control group ($P > 0.05$) but was significantly different to that of the 1 day, 5 day and 7 day CFR groups ($P < 0.01$). In addition, comparison of digestibility of the 3 day, 5 day, 7 day CFR groups suggests that this increases with increased duration of food limitation (Figure 2).

The mean body fat content of the control group was significantly higher than those of the 3 day, 5 day and 7 day CFR groups (LSD test, $P < 0.01$, Figure 3B) but markedly lower

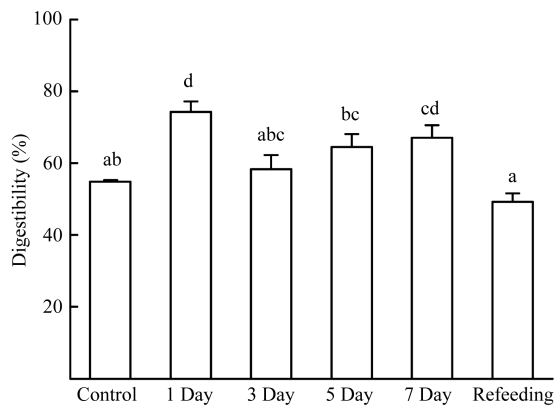


Figure 2 Differences in digestive efficiency in Chinese Bulbuls (*Pycnonotus sinensis*) subject to continual food limitation for 1, 3, 5 or 7 days and those subject to temporary food limitation for 7 days (TFR)

Different letters labeled above each bars indicate significant differences.

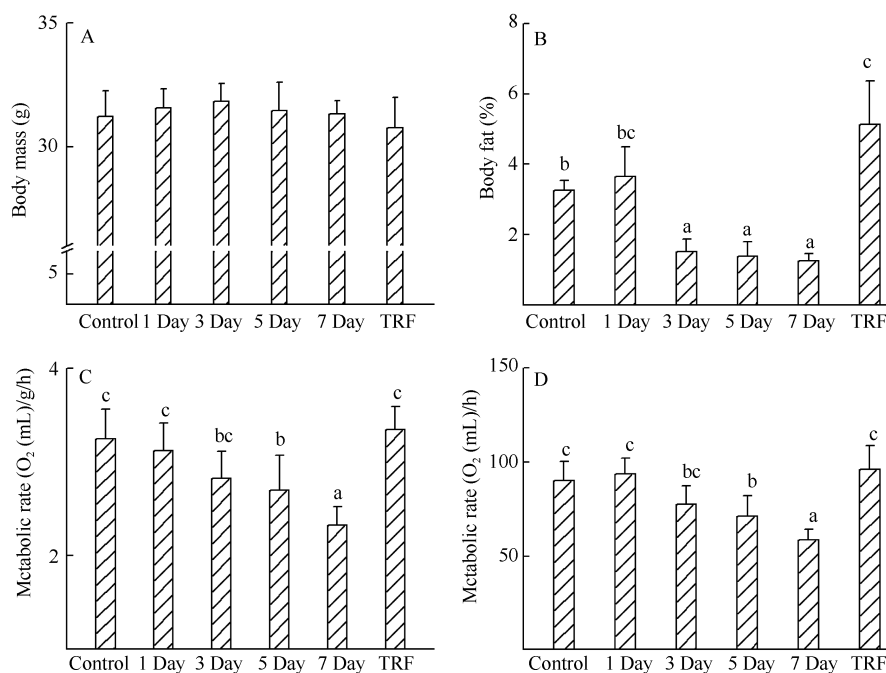


Figure 3 Differences in (A) initial body weight ($P=0.982$), (B) body fat, (C) total BMR and (D) unit BMR between Chinese Bulbuls (*Pycnonotus sinensis*) subject to either continual food restriction for 1, 3, 5 or 7 days and those subject to temporary food limitation for 7 days (TFR)

Different letters labeled above each bars indicate significant differences.

The total BMR of the 7 day CFR group was significantly lower than that of the control (LSD test, $t=2.717$, $P<0.05$) and the TFR groups ($P<0.05$, Figure 3D), but there were no significant differences between the BMR of the control and those of the 1 day, 3 day, and 5 day CFR groups (LSD test, $P>0.05$ in both comparisons). The total BMR of the 1 day CFR group was 12.6% higher than that of the control, but those of the 3 day, 5

than that of the TFR group ($P<0.05$), which was also significantly higher than that of the 3 day, 5 day and 7 day CFR groups ($P<0.01$, Figure 3B). Body fat content of control birds was 53.7%, 57.4% and 61.7% higher than that of the 3 day, 5 day and 7 day CFR groups, respectively, but 57.4% lower than that of the TFR group. As expected, comparison of the body fat content of the 1 day, 3 day, 5 day and 7 day CFR groups indicates a decline in body fat with increased duration of food restriction (Figure 3B).

BMR

Feeding regime and the energy intake levels significantly affected BMR. The 7 day CFR group had significantly lower unit BMR than both the control (LSD test, $t=2.463$, $P<0.05$) and TFR groups ($P<0.05$, Figure 3C), whereas no significant differences in unit BMR was found between the control and the 1 day, 3 day, 5 day, 7 day CFR groups (LSD test, $P>0.05$ in both comparisons). The unit BMR of the 1 day, 3 day, 5 day and 7 days CFR groups were 2.9%, 22.9%, 22.9% and 28.6% lower than that of the control group, respectively.

day and 7 day CFR groups were 22.2%, 20.7% and 30.0% lower than the control, respectively.

As expected, comparison of total and unit BMR values of the control, 1 day, 3 day, 5 day, and 7 day CFR groups shows a decline in BMR with increasing duration of food limitation (Figure 3C, D). Total and unit BMR of the TFR group was, however, essentially the same level as that of the control group.

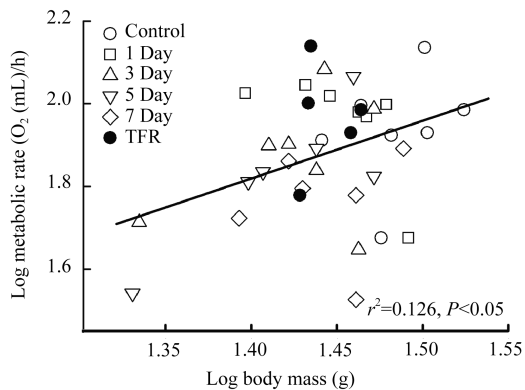


Figure 4 Relationship between log BMR and log body mass in Chinese Bulbuls (*Pycnonotus sinensis*) subject to either continual food restriction for 1, 3, 5 or 7 days or temporary food limitation for 7 days (TFR)

Log total BMR was positively correlated with log body mass ($r^2=0.126$, $P<0.05$, Figure 4).

Effects of temporary food restriction on body composition and digestive tract morphology Mass of internal organs

ANCOVA with mass as a covariate revealed marked differences in the dry mass of the lung, heart and spleen (lung, $F_{(5,33)}=2.560$, $P<0.05$; heart, $F_{(5,33)}=8.019$, $P<0.01$; spleen, $F_{(5,33)}=2.651$, $P<0.05$, Table 1) of different experimental groups. The control, 1 day CFR and TFR groups differed significantly in heart and lung dry mass compared to the 5 day ($P<0.01$) and 7 day ($P<0.01$) CFR groups (Table 1). Similar results were obtained for the brain, spleen, lung, and kidney but these differences were not always statistically significant. However, a comparison of the group means shows a tendency towards reduced organ mass with increasing duration of food restriction for all internal organs except the spleen (Table 1).

Table 1 Dry mass of various internal organs of Chinese bulbuls (*Pycnonotus sinensis*) subjected to either continual food restriction for 1, 3, 5 and 7 days, temporary food limitation for 7 days (TFR), or provided with food *ad libitum* (control)

Dry mass (mg)	Control	1 day	3 day	5 day	7 day	TFR	$F_{(5,32)}$	P
Brain	200.5±6.8	215.3±6.1	214.0±6.2	203.4±6.8	210.0±6.1	192.9±7.1	1.726	0.157
Heart	84.9±6.2 ^a	108.9±5.5 ^b	83.7±5.6 ^a	77.9±6.1 ^a	68.3±5.5 ^a	103.3±6.4 ^b	7.263	<0.001
Lung	48.2±3.4 ^{ab}	56.4±3.0 ^b	52.0±3.0 ^{ab}	49.0±3.4 ^{ab}	44.2±3.0 ^a	56.6±3.5 ^b	2.607	<0.05
Liver	333.8±20.5 ^{ad}	281.1±18.2 ^{ac}	285.4±18.6 ^{ac}	274.6±20.5 ^{ac}	268.8±18.2 ^c	394.2±21.4 ^{bd}	5.471	<0.001
Kidney	102.9±5.9	97.7±5.3	94.2±5.4	93.2±5.9	91.3±5.3	103.0±6.2	0.661	0.655
Spleen	8.5±2.3 ^b	10.5±2.0 ^{ab}	7.0±2.1 ^b	16.2±2.3 ^a	10.7±2.0 ^{ab}	15.5±2.4 ^a	2.829	<0.05
Stomach	138.9±9.6	142.5±8.6	134.5±8.7	123.8±9.6	126.3±8.6	157.3±10.1	1.554	0.201
Digestive tract	332.0±24.7 ^a	329.1±21.9 ^a	355.1±22.4 ^a	333.3±24.6 ^a	328.6±21.9 ^a	445.3±25.8 ^b	3.330	<0.05

Different superscripts in the same row indicate significant differences between groups.

Mass of digestive tract morphology

The stomach dry mass of the CFR groups were not significantly different to those of the control group (ANOVA, $F_{(4,29)}=2.465$, $P>0.05$, Table 1) but the digestive tract dry mass of the TFR group was significantly different to that of both the control and CFR groups (LSD test, $P<0.05$, Table 1). The digestive tract dry masses of the TFR group were 24.3%, 25.8%, 26.5%, 25.8% and 24.2% greater than those of the control and CFR. No other significant differences in digestive tract weight were detected. The stomach mass of birds in the 5 day and 7 day CFR groups was also significantly lighter than that of birds in the control and TFR groups but these differences ceased to be significant after stomach mass had been adjusted for differences in body mass (ANCOVA, Table 1).

Birds in the CFR groups had significantly lighter liver than those in the TFR and control groups (ANCOVA, $P<0.01$, Table 1). The liver dry mass of 1 day, 3 day, 5 day and 7 day CFR birds were 17.9%, 15.9%, 12.9% and 22.9% less than those of the control, whereas that of the TFR group was 12.4%, 28.2%, 26.4%, 32.2% and 32.5% greater than that of the control, 1 day, 3 day, 5 day and 7 day CFR groups respectively. Indeed,

comparison of the group mean values suggested that liver mass declined with increasing duration of food restriction.

Allometric relationships between log dry organ mass and log Mb (minus organ wet mass) were positive except for the brain, but only the dry masses of the liver, heart, and lungs were significantly correlated with body mass (Table 2). No other residual organ masses were significantly correlated with BMR residuals, but there were significantly positive correlations between BMR residuals and heart and lung dry mass residuals (Table 2).

DISCUSSION

Our results indicated that food restriction significantly affected body mass, fat content and BMR, all of which decreased significantly over a 7 day period. We did not find the effect of short-term food restriction following a hyperphagic period in the current study.

Effects of food restriction on body mass and fat reserves

Seasonal changes in body mass, especially in small passerine

Table 2 Linear regression statistics for allometric and residual correlations of log dry organ mass versus log body mass (minus wet mass of the organ), and dry organ mass versus metabolic rate (BMR) in Chinese bulbuls (*Pycnonotus sinensis*) from Wenzhou City, Zhejiang Province, China

	Liver	Heart	Brain	Lung	Kidney	Spleen	Stomach	Digestive tract
Allometric correlations:								
R^2	.169	.421	.001	.273	.105	.066	.011	.014
P	<.001	<.001	.944	<.001	<.05	.116	.534	.467
Slope	.497	.948	-.006	.551	.271	.793	.101	.136
Residual correlations:								
R^2	.050	.125	.007	.145	.088	.029	.138	.003
P	.171	<.05	.603	<.05	.066	.298	<.05	.732
Slope	.129	.242	-.023	.199	.124	.270	.185	.033

birds, are considered to be an essential adaptive strategy for survival (Cooper, 2000; Pendergast & Boag, 1973). More recent studies of migrating birds have shown that appreciable amounts of protein and fat may be stored to be used during migration (Bordel & Haase, 2000; Karasov & Pinshow, 1998; Liknes et al, 2014; Piersma & Lindström, 1997). Ecological field studies have revealed that migrants arriving at stopover sites recover body condition slowly for one to two days after which there is a much more rapid increase in body mass (Gannes, 1999; Hume & Biebach, 1996; Langslow, 1976; Lindström, 1995; Pierce & McWilliams, 2004; Rappole & Warner, 1976; Yong & Moore, 1997).

We found that bulbuls subject to restricted food intake for 7 day were 16.2% lighter than control birds. Body weight increased rapidly after food restriction was lifted but was still 6.4% lower than that of controls after 6 days. There was a significant linear relationship between body mass and the duration of food restriction and the body fat content of control birds was 53.7%, 57.4% and 61.7% higher than that of 3 day, 5 day and 7 day CFR birds, respectively.

Many studies have documented similar changes in fat levels in birds. For example, Karasov & Pinshow (1998) found that every gram of body mass lost by fasting blackcaps (*Sylvia atricapilla*) was mainly accounted for by fat loss. Pierce & Williams (2004) found that there was a significant difference in the body mass of *Zonotrichia albicollis* on a restricted diet compared to controls. This suggests that birds must rebuild fat reserves during migration stopovers. In any case, there is considerable evidence to suggest that body mass regulation is a very important adaptation to unpredictable food availability (Ekman & Hake, 1990).

Effect of food restriction on BMR

The regulation of energy metabolism is the main means by which birds maintain energy balance (Marsh & Dawson, 1989). The most prudent and tentative conclusion is that, in order to maintain energy balance, many resident and migratory birds lower their BMR in response to a decrease in ambient food. Such variation in BMR may reflect species, and environment specific, evolutionary survival strategies (McKechnie & Wolf,

2004). As in many previous studies, our results showed that the BMR of birds subject to food restriction was about 20% less than that of control group. There was also a trend toward declining BMR with increasing duration of food restriction. The BMR of TFR birds was essentially the same as that of the control group after food restriction ceased. Lindström (1995) also found that BMR decreased during food restriction and returned to the level of the control in a species of migratory passerine. Prinzing & Siedle (1988) reported that house martin (*Delichon urbica*) nestlings' metabolic rate decreased in response to food shortage. We also found that bulbuls subject to food restriction decreased their energy expenditure in order to maintain body mass. Within 8 days from the beginning of food restriction, a clear change in behavior was evident; bulbuls reduced movement to decrease heat loss. Schew & Ricklefs (1998) argue that there is an adaptive and active decrease in energy expenditure in response to food restriction. In addition, most bulbuls probably do face major changes in food abundance each year. Our results support the view that, 'despite the potential costs involved in exhibiting physiological flexibility (DeWitt et al, 1998), metabolic flexibility is a basic trait of a bird, not a result of evolutionary adaptation in populations in cold areas' (Klaassen et al, 2004).

Effects of food restriction on digestibility and digestive organs

In birds, the size of the digestive tract is likely to be limited by constraints associated with migration and/or flying. Change in digestive organs in response to temporary food restriction has been observed in many birds (Karasov & Pinshow, 2000) and may help maintain digestive efficiency and the body's energy reserves (Karasov & Pinshow, 2000; Starck & Rahmaan, 2003). Because a large digestive system requires increased energy to function, short-term food deprivation and migration may cause atrophy of the digestive tract (McWilliams & Karaso, 2001). The ability to reduce the size of the digestive tract is one of the ways by which birds successfully meet the conflicting physiological challenges of migration.

We found that bulbuls in the CFR groups had higher digestive efficiency than those in the control and TFR groups

(maximum 35.4%, minimum 6.6%). Digestibility of bulbuls in the 1 day and 7 day CFR groups was significantly higher than that of those in the control and TFR groups. However, there was no significant difference in digestibility of the control and TFR groups, probably because that of the TFR group returned to normal levels after food limitation ceased. There was no significant difference in the digestive tract and stomach dry mass of the CFR and control groups. Although, ANOVA indicated significant differences in the digestive tract mass of different experimental groups these disappeared when adjusted for differences in body mass. We conclude that bulbuls may enhance their digestive efficiency to compensate for reduced digestive organ mass during migration (Pierce & McWilliams, 2004) and when food is limited. This phenotypic plasticity and flexibility of the digestive tract is of vital significance in the life history of birds.

Effects of food restriction on body composition

Chinese bulbuls that were subject to food restriction had lighter livers than control birds, and liver, kidney, heart, lung mass generally declined with increased duration of food restriction. Similar changes in digestive organs associated with food restriction and migration have been observed in other birds like yellow-rumped warblers (*Setophaga coronata*), Semipalmated Sandpiper (*Calidris pusilla*), yellow-legged gulls (*Larus michahellis*), Songbird (*Zonotrichia albicollis*) (Lee et al, 2002; McWilliams & Karasov, 2005; Piersma & Gill, 1998; Pierce & McWilliams, 2004). This suggests that food deprivation has a profound effect on the body composition and digestive organs of birds. The liver is the largest and most important organ involved in metabolic activity in homeothermic animals (Villarin et al, 2003). Our results suggest that bulbuls slowly reduce the weight of the liver as the duration of food limitation increases. This may be an adaptive strategy for migration conferring the benefits of reduced metabolic rate and/or energy consumption. Interestingly, the size of the liver, kidneys, heart, spleen, stomach, digestive tract and lungs of the TFR group was greater than those of both the control and CFR groups, suggesting that organs necessary for flight may increase in size before departing a stopover site.

In conclusion, our results support the conclusions of previous research showing that body mass declines in response to food deprivation. When food is limited, bulbuls consume energy reserves, reducing body weight and body fat in order to maintain essential metabolic functions. In addition, they reduce energy consumption by reducing their basal metabolic rate and enhance digestive efficiency to compensate for reduced digestive organ mass. When adequate food resources once again become available all these parameters quickly return to normal. All these changes are of obvious adaptive benefit to a species that experiences marked variation in food availability. Along with the global climate warming, chinese bulbuls have showed a general adaptability to the shortage of food with a tendency to spread to the north.

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