

PHENOTYPIC FLEXIBILITY OF THE AVIAN GIZZARD: RAPID, REVERSIBLE AND REPEATED CHANGES OF ORGAN SIZE IN RESPONSE TO CHANGES IN DIETARY FIBRE CONTENT

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Accepted 7 September; published on WWW 28 October 1999

Summary

Evolutionary biology presumes that organ capacities match their natural loads. Therefore, in fluctuating conditions, organ systems are expected to show a reversible, repeatable and rapid phenotypic response that is directional and scaled. In this study, phenotypic responses of the gizzard of adult Japanese quail (*Coturnix japonica*) to experimental mismatches of load and capacity were tested by a series of diet-switching experiments, involving an increased content of non-digestible fibre (NDF) in the diet. The results of all experiments were in accordance with the predictions of the hypothesis that there is matching between loads and capacities. (1) The observed phenotypic responses are directional and scaled to the demands, i.e. increasing NDF elicits an increase in gizzard

size. When the proportion of NDF in the diet was raised from 1 % to 45 %, the gizzard was more than twice as large as in the control group. (2) Size responses were reversible, and reduced NDF was followed by a decrease of gizzard size. (3) Phenotypic responses could be elicited repeatedly in three successive trials. (4) Excess capacities were downregulated and insufficient capacities were upregulated. (5) The responses followed changes of loads with almost no time lag, with size changes measurable within 24 h.

Key words: Phenotypic transformation, gastrointestinal tract, nutrition, diet-switching, functional anatomy, quail, *Coturnix japonica*.

Introduction

From the perspective of evolutionary biology, the functional capacity of an organ should match the demands imposed upon it. Functional capacities in excess of the actual demands cost energy through maintenance of underutilized organ size and function. Insufficient capacity restricts the ability to exploit the full extent of resources, even if resources are available in unlimited supply. This hypothesis rests on two major assumptions. First, the energy budget of an individual is limited, and the maintenance of unutilized capacities costs a measurable/significant portion of its biosynthetic energy budget. Second, mismatching reduces fitness relative to individuals with optimized organ design, because of energetic costs. Therefore, individuals ought to respond to changes in the actual demands by adjusting their functional capacities (Diamond and Hammond, 1992; Diamond, 1998; Hammond, 1998; Weibel, 1998). The inferences from these ideas are as follows. (1) Phenotypic responses should be directional and scaled to changes in the demands in either direction. Excess capacities should be downregulated and insufficient capacities should be upregulated. (2) Responses are expected to be fully reversible. (3) Responses ought to be repeatable. (4) Phenotypic responses should follow environmental variation without a significant time lag.

Organs may, however, be designed with some reserve capacity, i.e. safety margins, to allow for instantaneous adjustments to daily fluctuations of resource availability (Diamond and Hammond, 1992; Konarzewski and Diamond, 1994; Hammond et al., 1994, 1996; Diamond, 1998; Hammond, 1998). Safety margins and phenotypic flexibility are complementary concepts of how an organism responds to fluctuations in the environment. Phenotypic flexibility of organ size and function begins when variation in resources or demands exceeds the reserve capacity, e.g. owing to seasonal changes of food availability or nutrient composition, or because of an increased metabolic demand, such as that experienced by birds during long-distance migrations. Presumably, phenotype safety margins are reorganized in concert with the up- or downregulation of functional capacities.

The gastrointestinal tract is a suitable organ system to study matches between loads and capacities because it is one of the major interfaces between the organism and its environment. Any fluctuation in the energy demands of an organism or in environmental resource availability imposes changing demands on the gastrointestinal tract to transport nutrients and energy. Phenotypic flexibility of the gastrointestinal tract in

response to changes in diet or organismal demand has been described in a variety of avian and mammalian species (Piersma and Lindström, 1997; Starck, 1999). Phenotypic transformations have been observed at the level of gross morphology, i.e. gut length and gizzard mass, and at the ultrastructural level, i.e. changes in microvilli number and length. Cellular turnover time, on which the ability to reorganize the phenotype of the gut depends, has been determined to range between a few days and 2 weeks (Lilja, 1987; Lilja and Amneus, 1987; Starck, 1996a,b).

This study focuses on the avian gizzard as a model system for investigating the directionality, scaling, dynamics and reversibility of phenotypic flexibility, and aims to relate laboratory-based results to ideas about the ecological role of phenotypic flexibility (Piersma, 1998; Piersma and Gill, 1998; Piersma et al., 1993, 1999; Starck, 1999). In the experimental design employed here, the organismal demands remained unchanged and the birds were kept under constant conditions. The loads to the gastrointestinal tract were changed by gradually increasing the non-digestible fibre (NDF) content of their diet. Increasing NDF content challenges the gastrointestinal tract by decreasing the energy content of the food while increasing its bulk. Predictions involve higher ingestion rates owing to the diluted energy content and increased gizzard size because of the increased need to grind and transport the more fibrous food.

Several implications of the hypothesis of matched loads and capacities are tested here in a series of feeding experiments: (1) gradually changing of loads tests for directionality and scaling of phenotypic responses; (2) repeated diet-switching tests for repeatability of phenotypic responses; (3) predictions about up- and downregulation are tested by repeated reversals of the challenge; (4) the time patterns of up- and downregulation are studied by repeated measurements of organ size, preferably of the same individual, by means of noninvasive imaging; (5) repeated and independently designed experiments test the reproducibility of phenotypic responses among and within experiments.

Materials and Methods

Animals

Japanese quail (*Coturnix japonica* f. dom.) were obtained from the Department of Agricultural Sciences, Division of Animal Production, Martin-Luther-University at Halle, Germany. Only males were used to exclude effects of sex and to avoid possible fluctuations and elevations of energy requirements due to egg production.

Food and feeding

Standard food (dry matter: 22.6% crude protein, 4.7% lipids, 12.2% ash, 3.8% sugar, 3.3% fibre, 45.2% starch, 4% calcium, 0.9% phosphate) was obtained from the Department of Animal Production of the University Stuttgart-Hohenheim, Eningen, Germany. Non-digestible fibre (NDF; oat beards) was purchased from a commercial mill in Lahr, Germany. Experimental diets contained 15%, 30%, 45%, 60% and 70%

NDF by mass. During all phases of the study, food and water were offered *ad libitum*.

Animal care

Quail were kept in standardized stainless-steel wire boxes (30 cm×30 cm×20 cm) according to European Community standards at an average temperature of 22 °C, 50% air humidity, and a 14 h:10 h light:dark regime.

Dissections and morphometry

Animals were killed by cervical dislocation. Macroscopic dissections and organ size measurements were performed immediately thereafter. Organs (gizzard, glandular stomach, liver) were weighed using a laboratory scale (precision 0.01 g). Gizzard and glandular stomach length was measured using a slide caliper (precision 0.05 mm). The remaining carcasses were stored at -30 °C for further analyses.

Ultrasonography

Ultrasonography was performed on live birds using an ophthalmological ultrasonography device (I³ Innovative Imaging Inc., Sacramento, California), equipped with a 10 MHz sector scanner in B-mode (scan angle, 52 °; scan speed, 28 frames s⁻¹; frequency, 10 MHz (90 dB); resolution, 0.15 mm axially, 0.2 mm laterally; image depth, 45 mm; gray scale, 256 shades; cross vector scale, calibrated to 1550 m s⁻¹). The applicator surface was concave. Transcutaneous ultrasonic imaging was performed using ultrasonic gel for coupling the applicator to the surface of the animal. The animals received no prior treatment. Ultrasonography scans were stored as TIFF files and SigmaScanPro 4.0[®] (Jandel Scientific) was used for image analysis and morphometry. Ultrasonographic measurements were calibrated with morphometric measurements from dissections.

Feeding experiments

Three independent feeding experiments were performed on 60, 140 and 50 quail, respectively. The experiments were designed to allow for testing of the results of each independent experiment for between-experiment reproducibility.

Experiment 1

Test of directionality and scaling of phenotypic responses of the gizzard to NDF diet

60 quail were adjusted to the standard diet for 4 weeks. Then, ten quail were randomly assigned to each of six diet groups of increased fibre content (0%, 15%, 30%, 45%, 60% and 70% NDF). Quail were fed the experimental diet *ad libitum* for 14 days. Body mass and food intake were measured on a daily basis. Birds were killed after 2 weeks, and their organs were studied for morphometric changes.

Experiment 2

Test of the reversibility and repeatability of phenotypic responses of the gizzard and the glandular stomach to 45% NDF diet

In this experiment, 140 quail were adjusted to the standard

diet for 4 weeks (days -28 to 0). Then, 70 quail were randomly assigned to the experimental group and 70 to the control group. At day 0, the experimental group was switched to a diet containing 45% NDF. After 14 days, 10 birds of each group were killed, and their internal organ morphometry was studied. The diet of the remaining experimental birds was switched back to the standard diet until day 28, when a further 10 birds from each group were killed. Diet-switching from standard to experimental diet and back was repeated again three times. During the entire experiment, the control group was fed the standard diet.

Experiment 3

Study of the time course of structural reorganization of the gizzard and glandular stomach

50 quail were allowed to adjust to the standard diet for 4 weeks. At day 0 of the experiment, all quail were switched to a diet containing 45% NDF. After 14 days, quail were switched back to the standard diet for another 14 days. The entire feeding trial lasted 28 days, equivalent to one diet-switching period in experiment 2. Five quail were killed before diet-switching and then, during the course of the experiment, five quail were killed at intervals of 1–5 days, and the morphometry of their internal organs was studied. In addition, ultrasonographic measurements of the gizzard size were performed at intervals of 1–3 days (five measurements per individual animal and session).

Statistics

Values are given as mean \pm s.d. The sample size that would be necessary to detect differences among means at $P=0.01$ was determined following Sokal and Rohlf (1995). A general linear model (SAS 6.12) was used to test for effects of food and individual. Food was treated as a fixed effect, while individual was a random effect. The Ryan–Einot–Gabriel–Welsch multiple-range test (REGWQ, SAS 6.12) for preplanned comparisons among means was performed at $\alpha=0.001$. The REGWQ controls for maximum experiment-wise error rate under any complete or partial null hypothesis, and is currently the most powerful of a series of multiple-range tests available in SAS 6.12. For scaling of changes in organ size to changes in food, the data from experiments 1 and 3 were approximated by nonlinear functions using the Marquard–Levenberg algorithm. To test for the reproducibility of results within and between experiments, an estimate of the reproducibility (r), which is the intraclass correlation coefficient, was calculated by a mixed-model analysis of variance (ANOVA) approach, which included the factors experiment and food:

$$r = \frac{s^2_A}{(s^2 + s^2_A)}, \quad (1)$$

where s^2_A is the among-groups variance and s^2 is the within-groups variance. Reproducibility was calculated within and between experiments by using 'experiment' and 'food' as factors (Lessells and Boag, 1987).

Results

Directionality and scaling of phenotypic flexibility (experiment 1)

Body mass

The body mass of control birds averaged 141 ± 2 g ($N=10$) over the experimental period. An increase in body mass occurred from 138 ± 10 g at day 1 of the experiment to 142 ± 8 g at day 14, but this increase was not statistically significant. Quail in all experimental groups except 15% NDF declined in body mass after diet-switching (Fig. 1A). In the 30% NDF and 45% NDF groups, the decline in body mass was moderate and levelled off after 5–6 days at 134 ± 11 g for the 30% NDF group and 126 ± 9 g for the 45% NDF group. At the end of the experiment (day 14), birds in both groups had regained some body mass. At that time, the 30% NDF group averaged 138 ± 11 g, and the 45% NDF group averaged 128 ± 9 g. Quail feeding on 60% NDF lost body mass continuously throughout the entire experiment, decreasing to 116.1 ± 8 g at day 14. In the 70% NDF diet group, loss of body mass was so great that these birds were removed from the experiment after 12 days when body mass averaged 98 ± 17 g. Five of these quail were used for

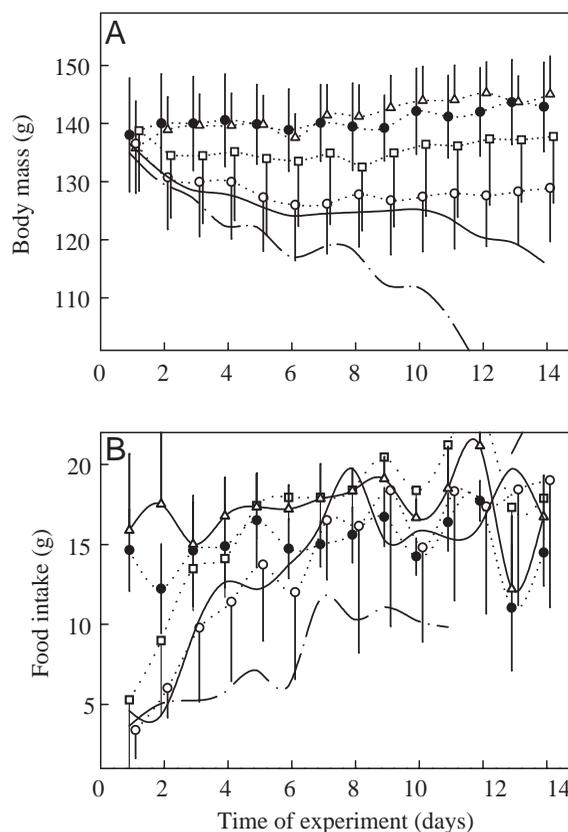


Fig. 1. (A) Body mass changes in response to diet-switching. On day 0, food was switched in all groups except for controls. (B) Food intake in controls and experimental groups when switched to the non-digestible fibre (NDF) diet. $N=10$ animals except for 70% NDF ($N=5$ animals). Values are given as means \pm s.d. Filled circles, control; open triangles, 15% NDF; open squares, 30% NDF; open circles, 45% NDF; solid line 60%; dash-dot line 70% NDF, respectively.

morphometry. The other five were re-fed and regained the prior average body mass within a week.

Food intake

On average, control birds ingested 15.3 ± 1.8 g food per day (Fig. 1B). Ingestion rates varied so much between days that the differences became significant when days 12 and 13 were compared (standard diet, REGWQ-test, $P=0.01$). However, there was a high degree of autocorrelation among food intake data for successive days. Quail feeding on the 15% NDF diet increased their daily food intake immediately after diet-switching by 1.6 g to 16.9 ± 1.8 g. In all other groups, food intake dropped immediately to values between 5.3 ± 2.5 g per day in the 30% NDF group and 3.4 ± 2.0 g per day in the 70% NDF group. After 3 days in the 30% NDF diet group, 6 days in the 45% NDF diet group (Fig. 1B) and 7 days in the 60% NDF diet group, birds resumed the average food intake of 16 g per day. Quail on 70% NDF diet increased their intake rates to 5–10 g per day, but could not re-establish the food intake rates of the controls. Resumption of the control ingestion rate coincides with the stabilization of body mass at day 6 in the 45% NDF diet group and day 3 in the 30% NDF diet group.

Gizzard size

After 14 days on experimental diet, the birds were killed and gizzard mass and length were measured. A gradual increase in gizzard mass and length with increasing fibre content was noted (Fig. 2A). The 15% NDF group of quail had gizzards that were 1.6 g heavier (3.67 ± 0.59 g) than in the 0% NDF group (2.04 ± 0.16 g). An NDF content of 30% caused the mass of the gizzard to increase to 5.07 ± 0.61 g. Between 30% and 45% NDF, the responsiveness reached an asymptote at a mass of 5.64 ± 0.97 g. Above 45% NDF, no further mass increase could be elicited by increasing the fibre content of the food. The responsiveness of gizzard mass was most closely approximated by a nonlinear regression. The asymptotic gizzard mass levelled off at 45% NDF, at a mass almost three times (275%) that found before diet-switching. The response curve can be described by a sigmoidal three parameter model of the form:

$$M = \frac{a}{1 + e^{-\left(\frac{x-x_0}{b}\right)}}, \quad (2)$$

where M =mass, x is % NDF and a is asymptotic size (empirical value 5.6 g); b is a constant and is estimated to be 11.1 ± 1.26 (mean \pm S.E.M.) and x_0 is estimated to be 6.8 ± 1.18 (mean \pm S.E.M.) ($r^2=0.84$; $P<0.0001$ for estimated parameters b and x_0). A *post-hoc* comparison of gizzard mass showed that control, 15% and 30% NDF diet groups differed significantly from each other in gizzard mass. In accordance with the lack of further response, no significant differences could be detected between 30%, 45%, 60% and 70% NDF diet groups (Fig. 2A; REGWQ-test for multiple comparisons among means, $\alpha=0.001$). The phenotypic response of the gizzard to increasing NDF content is limited to a maximum gizzard mass of 5.6 g at

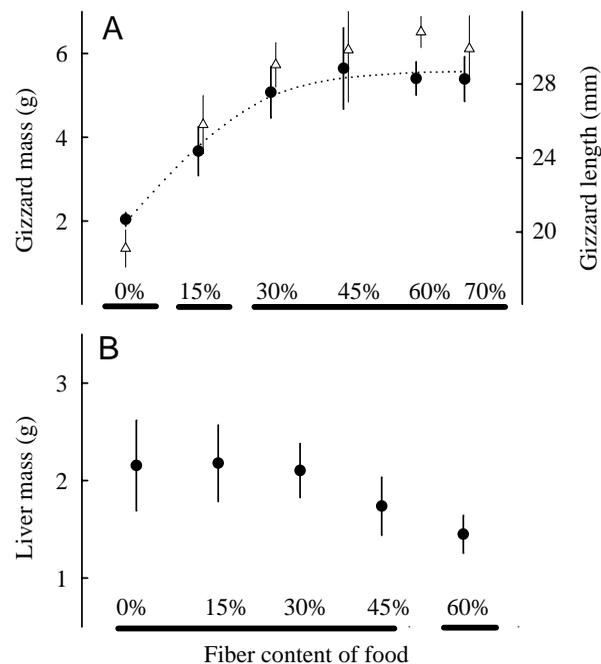


Fig. 2. (A) Response of gizzard mass and length to increasing non-digestible fibre (NDF) content of diet. Values are means \pm S.D., $N=10$ except for 70% NDF ($N=5$). Filled circles, gizzard mass; open triangles, gizzard length. REGWQ-test for differences among means; means enclosed by the range of one line are not significantly different at $\alpha=0.001$. (B) Response of liver mass.

45% NDF. Increasing the NDF content of food beyond this could elicit no further increase in gizzard mass. Gizzard length showed an identical response (Fig. 2A).

Liver mass

Quail feeding on 60% NDF had significantly smaller livers than birds of the other groups (REGWQ-test for multiple comparisons among means, $\alpha=0.001$). There was no consistent pattern of liver mass decrease in relation to increased fibre content of the food (Fig. 2B).

Reversibility and repeatability (experiment 2)

Food intake

The food intake showed the same pattern as in experiment 1. On average, control birds ingested 12.4 ± 2.8 g food per day. Daily fluctuations of intake rates between 4.7 g and 17.6 g per day could not be explained by experimental effects (Fig. 3A). Birds that were switched to an experimental diet of 45% NDF immediately reduced their food intake rates to 0.8–3 g per day. Thereafter, their food intake increased steadily, and after 6 days, the quail achieved ingestion rates of approximately 17 g per day (Fig. 3B). Intake rates on standard diet did not exceed 25 g per day. Three repeats of diet-switching produced approximately the same pattern of intake rates (Fig. 3B).

Gizzard size

Mass and length were measured to establish gizzard size. In birds, feeding on standard diet (control), the gizzard averaged

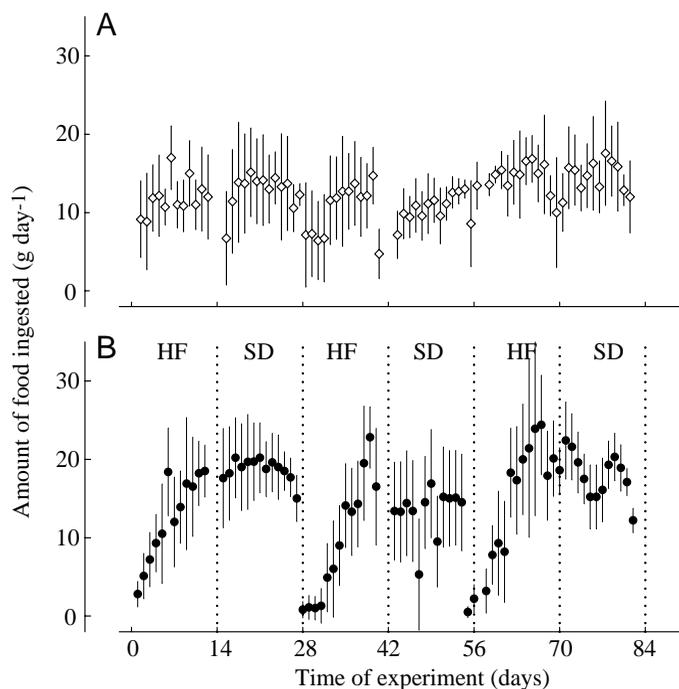


Fig. 3. Daily food intake of quail during an experimental period of 12 weeks. Values are means \pm s.d., $N=10$ for each data point. (A) Quail feeding on the standard diet (control). (B) Quail alternating between a 45 % NDF diet (HF) and a standard diet (SD).

2.6 \pm 0.3 g and 19.5 \pm 1.2 mm. During the 12 weeks of the experiment, the gizzard mass and length did not change in the control group (Fig. 4), except for day 70 when gizzard size differed from day 0. In the group that was challenged by a 45 % NDF diet, gizzard mass had increased to 221 % of the original mass (5.75 \pm 0.8 g), and gizzard length had increased to 144 % of its original value (28.0 \pm 1.3 mm) after 14 days. These values are in good accord with the results for 45 % NDF diet from the previous experiment. Switching back to standard diet, the size of the gizzard decreased to an average mass of 3.36 \pm 0.36 g and average length of 22.4 \pm 0.7 mm (Fig. 4). The downregulated gizzard was significantly larger (by approximately 30% for mass and 15 % for length; REGWQ-test, $\alpha=0.001$) than at the beginning of the experiment, and larger than in the control groups at any time during the experiment. Repeated and reversed diet-switching elicited repeated up- and downregulation of gizzard size. The magnitude of the increase and decrease of gizzard size was identical for the first and second trial, but smaller in the third trial (mass 5.6 \pm 0.7 g, not significantly different; length 26.5 \pm 1.6 mm; REGWQ-test, $\alpha=0.001$).

Time course of organ size changes (experiment 3)

Body mass

This experiment was designed as an independent repeat of the previous experiment. Up- and downregulation was elicited only once. Body mass followed the same pattern as described in experiment 1. After switching from standard diet to 45 % NDF diet, body mass declined for 6 days and then stabilized

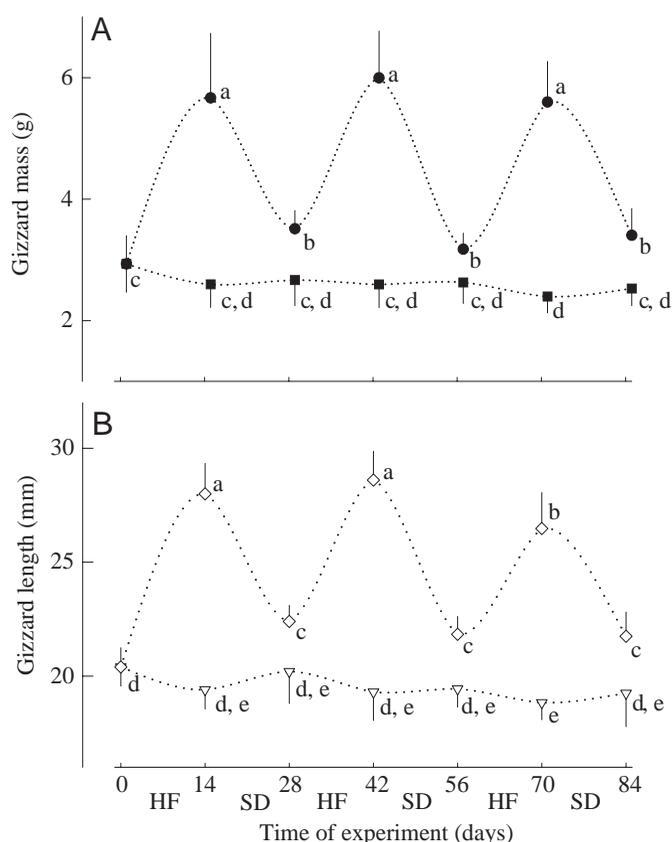


Fig. 4. Repeatability and reversibility of change in gizzard mass (A) and length (B) in response to 45 % NDF diet. Values are means \pm s.d.; $N=10$ for each data point. Filled squares, gizzard mass control; filled circles, gizzard mass experimental groups; open triangles, gizzard length control; open diamonds, gizzard length experimental groups. Quail alternated between HF (45 % NDF diet) and SD (standard diet). REGWQ-test for differences among means; means in the same curve labeled with different letters are significantly different ($\alpha=0.001$).

at 128 \pm 11 g. Switching back to standard diet, body mass increased again to an average of 132 \pm 12 g.

Gizzard size

Gizzard mass and length were measured using standard dissection techniques. Gizzard length was also measured using ultrasonography. Ultrasonographic measurements had to be multiplied by a factor of 1.2 to account for systematically biased measurements. With this correction, the differences between length measurements from ultrasonography and dissections could not be differentiated statistically and both types of data were pooled for further analysis. Because ultrasonography renders only length data, the data presentation focuses on gizzard length, with gizzard masses (from dissections) given in parentheses. Gizzard length was 20.4 \pm 1.64 mm (mass 2.7 \pm 0.4 g) before switching to 45 % NDF diet. After diet-switching, gizzard size initially increased almost linearly by 2 mm per day to reach an asymptotic length of 27.8 \pm 0.9 mm (mass 5.7 \pm 0.86 g) 6 days after diet-switching.

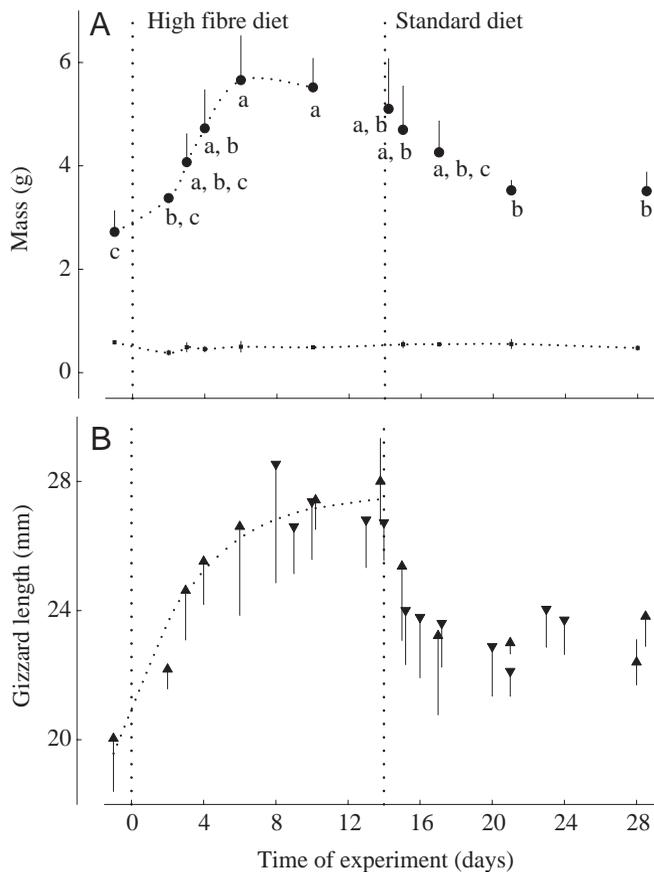


Fig. 5. Time course of changes in (A) gizzard mass (filled circles) and glandular stomach mass (filled small squares) and (B) gizzard length in response to a 45% NDF challenge. Length data are combined from gross morphological morphometry (\blacktriangle) and ultrasonographic measurements (\blacktriangledown). Perpendicular dotted lines indicate diet switches from standard to 45% NDF (day 0) and back from 45% NDF to the standard diet (day 14). Values are means \pm S.D.; $N=5$ for gross morphology data points and $N=25$ for ultrasonography data points. REGWQ-test for differences among means; means labelled with different letters are significantly different ($\alpha=0.001$).

This represented a 40% increase in length and a 110% increase in mass. The gizzard remained enlarged until the diet was switched back to standard diet at day 14, when gizzard length started to decline immediately at a rate of 1–2 mm per day. After 5 days, the gizzard reached the downregulated size of 22.4 ± 0.7 mm (mass 3.5 ± 0.37 g). As in experiment 2, the downregulated size was slightly larger (length 22.4 ± 0.7 mm; mass 3.5 ± 0.37 g) than before the experiment (length 20.04 ± 1.64 mm; mass 2.7 ± 0.4 g).

In this experiment, the mass of the glandular stomach was also recorded. Interestingly, the glandular stomach mass changed little in response to changes in NDF content of food but the value on day 2 was different from the pretreatment value (REGWQ-test, $\alpha=0.001$; Fig. 5A). The glandular stomach mass averaged 0.5 ± 0.08 g over the entire period of the experiment.

Reproducibility

A mixed-model ANOVA was performed with 'diet' and 'repeat' as factors to calculate the within-experiment reproducibility, and with 'diet' and 'experiment' as factors for among-experiment reproducibility. The reproducibility was estimated by dividing the among-groups mean squares by the combined mean square of among-group and within-group mean square, thus providing an F -test of whether differences between experiments are greater than the difference estimated from measurements. For repeated challenge with 45% NDF within experiment 2, a reproducibility of 0.95 was calculated. Differences among upregulated and among downregulated repeats were not significant, supporting the within-experiment reproducibility. A comparison of upregulation among experiments 1–3 also rendered a high reproducibility value of 0.97. Differences among upregulated gizzard sizes in different experiments were not significantly different from those estimated from measurement error.

Discussion

Matching/mismatching

In the feeding trials, increasing NDF changed the load to the gizzard by lowering the energy content (i.e. need to process more food) and increasing coarseness of the food. The limit to digestive capacity appeared to be the ability of the gizzard to process a given amount of food per day. The immediate decline in body mass after diet-switching indicates a mismatch between digestive load and digestive capacity. Quail required 6 days to upregulate their gut capacities to match the increase in load.

The following results of this study are consistent with the inferences generated from the hypothesis regarding the matches of loads and capacities. First, gradually increasing the load to the gizzard elicits an enlargement of the gizzard (mass/length) that is scaled to increasing NDF content of the food by an asymptotic function. This response reaches an upper limit of gizzard mass of 5.6 ± 0.9 g. It cannot be determined from the experiments whether the limit is ultimately set by the inability of the gizzard to increase above that size or by upstream (i.e. intake rates, capacity of the oesophagus and crop) or downstream (the capacity of the intestine) bottlenecks. The observations that quail decreased food intake rates after diet-switching and that quail on the control diet were already feeding at a maximum daily ingestion rate of 17–20 g suggest that quail always eat the maximum they can digest. The inability of the gizzard to increase in mass beyond 5.6 g limits the capacity of the quail to cope with food containing more than 45% NDF. The continuous loss of body mass when fed food containing more than 45% NDF was caused by limitations of gizzard performance and the inability to elevate intake rates much above 20 g per day. Second, repeated and reverse diet-switching elicited repeated up- and downregulation of gizzard size. When the diet was switched to high NDF, food intake rates decreased, but the reverse switch from 45% NDF to standard diet did not elicit a reduction in

ingestion rate, which might have been expected as a result of feeding on a better quality diet. Up- and downregulation of gizzard size is, therefore, independent of food intake rates and is probably induced by diet composition. The within- and between-experiment reproducibility was very high. These are the first data that show an instantaneous upregulation of undercapacity and downregulation of overcapacity on a gross morphological level. The observed downregulation when a load is released supports the idea that the maintenance of large capacities costs a considerable amount of biosynthetic energy. Interestingly, the gizzard size did not return to the original size after the first challenge, but remained enlarged. The larger residual size compared with that of unchallenged controls was consistently measured within and between experiments. Three explanations may be offered to explain the large residual value. (1) There may be differential downregulation, i.e. only the 'expensive' musculature is adjusted to demands, while the 'cheap' connective tissue and vascularization remains larger. An analogy may be seen in the female mammalian uterus which, during pregnancy, increases in size through hyperplasia and hypertrophy of smooth muscle cells of the myometrium and changes in the vessels. As the uterus returns towards its normal size after parturition, the muscle cells rapidly diminish, but the blood vessels retain their enlarged phenotype. The uterus does not return to its original size (Naaktgeboren and Slijper, 1970; Bargmann, 1977; Bloom and Fawcett, 1986). (2) The residual size of the gizzard may depend on the probability of another challenge and the costs of maintaining a large gizzard size. It can be hypothesized that, within a biologically meaningful range of energetic costs of gut maintenance and the probability of another challenge, it does not pay to downregulate gizzard size to the initial level (M. Konarzewski, personal communication). Results from experiment 3 can exclude the possibility that the large residual size was due to insufficient time for the gizzard to return to original size. (3) The observed residual size of the gizzard is about the same as after a 15% NDF challenge (Figs 2A, 4, 5). It is possible that a response to 15% NDF is within the 'safety margins', and that downregulation of gizzard size after a major challenge first returns to the upper level of the safety margin before it ultimately returns to the original size. Third, the phenotypic responses of the quail gizzard followed the changes in load almost instantaneously. Changes in gizzard size can be measured within 24–48 h of diet-switching, and upregulation was completed within 6 days of diet-switching. The same dynamics have been observed for downregulation. A response has been determined 24 h after removing the NDF challenge and switching back to standard diet. 4 days later, downregulation renders significant differences as compared to peak values (SAS glm; comparison of least squares; $P < 0.028$). Downregulation is accomplished within 6 days after diet-switching. Again, gizzard retains some residual size above the size before the challenge. The achievement of up- and downregulation within 6 days shows clearly that residual size is not an artefact of time constraints on downregulation. The glandular stomach responded little to the NDF challenge and

two different interpretations can be provided for this minimal response. Either the variation in NDF did not change the load imposed on the stomach, or the glandular stomach is unresponsive to changes in digestive demand. The correct solution will only be found after systematic trials of different nutritional challenges.

In summary, the phenotypic responses of the gizzard of the quail to an NDF challenge are in accordance with all predictions arising from the hypothesis that loads and capacities should match. Thus, (1) the observed phenotypic responses are directional and scaled to the demands; (2) the responses are reversible and can be elicited repeatedly; (3) excess capacities are downregulated and insufficient capacities are upregulated; and (4) the responses follow the variation in loads with (almost) no time lag. The response time of the gizzard is short enough to change the phenotype before mismatching of loads and capacities induces energy deprivation and potential starvation.

Comparative information

Comparative information about organ size changes is limited. In a recent review of published data (Starck, 1999), I have shown that phenotypic flexibility of the gastrointestinal tract has been reported from 31 bird species, nine mammals, one snake, one frog and one fish. However, almost all studies only consider increases in organ size and function, and decreases have been ignored. In addition, many of these studies, particularly those of birds, do not exclude the possibility of circannual endogenously controlled changes in organ size and phenotypic responses to changes in environment. Examination of the dynamics, direction and repeatability of such transformations were neglected. Clear experimental evidence for directional, repeatable and reversible structural flexibility comes from studies of Japanese quail, laboratory mice (Hammond et al., 1994, 1996; Hammond, 1998; Konarzewski and Diamond, 1994) and snakes (Secor and Diamond, 1994, 1995, 1998; Secor et al., 1994; Starck and Burann, 1998a,b). Savory and Gentle (1976a,b) presented indirect evidence that changes in diet may elicit reversible size changes in the gastrointestinal tract of Japanese quail. Starck and Kloß (1995) and Kloß (1996) studied the responsiveness of the quail gut and elicited considerable size changes according to changes in a variety of macronutrients. Although different feeding conditions were applied, the response to a 5% NDF diet reported in these papers fits the data presented in this paper: a 5% NDF diet elicited a 30% increase in gizzard size (length 25.2 ± 2 mm) compared with the control (length 19.2 ± 0.7 mm). Measured by ultrasonographic morphometry, the gizzard size of knots (*Calidris canutus*) undergoes changes of 40–50% in about the same time-frame as that observed in quail when challenged appropriately (M. Dietz, A. Dekinga and T. Piersma, personal communication).

Time course

No data about the time course of phenotypic transformations

of the avian gizzard have come to my attention. Limited data are available concerning upregulation of the small and large intestines. Kehoe et al. (1988) reported a size increase in the gut morphology of mallards within 5 days of diet-switching. Indirect evidence from studies that investigated intestinal cell proliferation and epithelial turnover times supports fast phenotypic transformations in a variety of bird species (Imondi and Bird, 1966; Lilja, 1987; Lilja and Amneus, 1987; Kloß and Starck, 1993; Starck and Kloß, 1995; Starck, 1996a,b). Depending on the size and age of a bird, cellular turnover times range between 4 days and 2 weeks. Such relatively short times would allow for a 20% cellular turnover within 1–3 days. Although the cytological data on intestinal mucosa turnover time cannot be compared directly with the data on gizzard size changes, they do indicate a time-frame for organ size changes close to that observed in this study.

Ecological implications

Phenotypic flexibility, i.e. the adjustment of organ size and function to the actual demands, has considerable ecological importance for birds living in fluctuating environments. Understanding the degree of phenotypic flexibility, its dynamics and limitations, will improve our understanding of the ecological setting of a species. For example, when seasonal variation in fibre content occurs in natural diets, it is of benefit to the bird in the natural environment when the gut adjusts flexibly to changes in diet quality. Recent studies on long-distance migrating birds have pointed to a surprising phenotypic flexibility of the muscular stomach (Piersma et al., 1993, 1999; Piersma, 1994, 1998; Battley and Piersma, 1997; Piersma and Gill, 1998). The present study amends and extends such ecological research, because it investigates patterns and mechanisms of phenotypic transformation that cannot be studied in such detail in the field. This study is the first to demonstrate that phenotypic transformations of the avian gizzard occur as rapid, repeatable and reversible responses to changes in external conditions. An implication of ecological importance arising from these results is the breakdown of protein and the availability of amino acids resulting from gizzard atrophy. For migrating birds, these amino acids may be used as a source of energy to fuel migration, as speculated by Piersma (1998) and Piersma et al. (1999). However, to assess the importance of such factors in an energy budget the patterns and processes of organ size changes and protein turnover need to be elucidated in more detail.

Implications for evolutionary morphology

In the late 19th century, observations of phenotypic transformations of the avian gizzard in response to changing feeding regimes fuelled a discussion about Lamarckian and Darwinian principles of evolution (Starck, 1999). Schmalhausen (1949) was the first to position phenotypic flexibility in a consistent evolutionary theory as functional modifications, functional adaptations or accommodation. To Schmalhausen (1949), the study of phenotypic flexibility and its reaction norms could improve our understanding of how the

degree of functional modifications/individual adaptability may shape the processes of speciation and evolutionary diversification (Schmalhausen, 1949). We are still far from understanding how phenotypic flexibility and evolutionary processes are integrated, especially if we consider the vertebrate gastrointestinal tract. However, an increased knowledge of the degree of phenotypic flexibility, its mechanisms and limitations will provide a baseline for understanding how the flexibility of organ size and function integrate with the ecology of a species.

I thank Sybille Koch and Karsten Rotthowe for help in the laboratory. I am grateful to Barbara Helm, Maurine Dietz and Theunis Piersma for comments and suggestions on an earlier version of this manuscript. The study was supported by grants STA 345/2-2 and STA 345/5-1 from the German Research Council (DFG).

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