The Journal of Experimental Biology 206, 1887-1897
© 2003 The Company of Biologists Ltd
doi:10.1242/jeb.00372



# Phenotypic flexibility of structure and function of the digestive system of Japanese quail

J. Matthias Starck[1,*] and Gamal Hasan Abdel Rahmaan[1,2]

[1]*Institute of Zoology and Evolutionary Biology, University of Jena, Erbertstraße 1, D-07743 Jena, Germany* and [2]*Institute of Zoology, Assiut University, Assiut, Egypt*

*Author for correspondence at present address: Department of Biology II, University of Munich, Luisenstrasse 14–16, D-80333 Munich, Germany (e-mail: starck@zi.biologie.uni-muenchen.de)

*Accepted 13 March 2003*

## Summary

**Organisms adjust their phenotype to fluctuating conditions of the environment and to changing internal demands. We report flexible responses of the gizzard and the small intestine of Japanese quail to a high-fibre diet. Switching from a standard diet to a high-fibre diet results in a highly significant increase in gizzard size, intestine length, mucosal surface, thickness of the intestinal muscular layer and vascularization of the mucosa. After diet switching, increased or decreased gizzard size results from changes in cell size, i.e. smooth muscle cell hypertrophy and hypotrophy, respectively. Increased cell proliferation is not the cause of increase in gizzard size. In the small intestine, however, we found elevated levels of cell proliferation after diet switching and conclude that increased capacity (upregulation) of the small intestine is based on increased rates of mitosis in the intestinal crypts. It is highly probable that elevated levels of cell proliferation in the crypts are balanced by elevated levels of cell extrusion at the tip of intestinal villi. The lipid contents of the liver were reduced, indicating that lipid stores in the liver were mobilized to fuel the flexible response of the gastrointestinal tract. During changes of organ size in response to changes in food composition, resting metabolic rate was not altered.**

Key words: Diet switching, dietary fibre content, cell proliferation, resting metabolic rate, hypertrophy, hyperplasia, Japanese quail, *Coturnix japonica*, gizzard, small intestine, liver.

## Introduction

Many organisms experience considerable seasonal changes in environmental conditions and internal physiological demands. Environmental changes include fluctuations in food availability and nutrient composition. Internal demands may change because of increased energetic requirements during reproduction or long-distance migration, and reduced energetic needs during hibernation (McWilliams and Karasov, 2001; McNab, 2002). The organisms' flexible responses to such changed conditions ought to be fast, reversible and repeatable (Piersma and Lindström, 1997; Starck, 1999a; McWilliams and Karasov, 2001; Hume et al., 2002; Piersma, 2002; Piersma and Drent, 2003). Three levels of response have been discussed. (1) Safety margins (Diamond and Hammond, 1992; Konarzewski and Diamond, 1994; Hammond et al., 1994, 1996; Diamond, 1998; Hammond, 1998), which are thought to present an instantaneous buffer against short-term environmental or internal changes, and are maintained at the cost of unused capacity. However, empirical proof of safety margins is difficult and the concept has consequently been challenged (e.g. Garland, 1998). (2) Physiological acclimatization/acclimation, i.e. up- and downregulation of a physiological function in response to changes in an environmental/experimental parameter. Physiological acclimation/acclimatization is fast and it may provide the required capacity within hours, but only within the framework of the existing physiological performance. (3) Phenotypic flexibility is the induced modification of a morphological or physiological trait (Travis, 1994; Piersma and Lindström, 1997; Hume, 1998; Starck, 1999a,b; Piersma and Drent, 2003) in response to long-term changes in conditions. Phenotypic flexibility is relatively slow because it involves biosynthesis of new enzymes or cells. It resets organ size and function together with the safety margins at a new functional range. The three levels of phenotypic response overlap to some degree, but differ in response time.

The phenotypic flexibility of the avian digestive system has been studied earlier and it has been shown that intestine size and function respond to a variety of nutritional factors as well as to changes in internal demands (Savory and Gentle, 1976a,b; Karasov, 1996; Piersma and Lindström, 1997; for reviews, see Starck, 1999a; McWilliams and Karasov, 2001). For the small intestine, a response time of just a few days has been suggested based on measurements of the tissue turnover time, i.e. 100% replacement of all cells of a tissue. Depending on the age and segment of the intestine, tissue turnover times range from 3 to 17 days (Imondi and Bird, 1966; Uni et al., 2000; Lilja, 1987;

Lilja and Amneus, 1987; Starck, 1996a,b). It has also been shown that the gizzard of birds adjusts to changes in diet composition (Spitzer, 1972; Starck, 1999b; Dekinga et al., 2001). After switching the diet from soft to hard food, the gizzard doubled in size within 6 days in Japanese quail (Starck, 1999b) and within 6–8.5 days in red knots *Calidris canutus* (Dekinga et al., 2001).

Here, we investigate the processes at the cell and tissue levels that underlie changes in organ size response to changes in dietary fibre composition, and also whether there are changes in resting metabolic rate associated with up- and downregulation of the gastrointestinal capacity. Although flexible responses to feeding a high-fibre diet have been reported previously, nothing is known about the underlying cellular processes, i.e. cell proliferation or increase in cell size. Flexible responses would vary considerably in complexity and timing depending on whether they were based on proliferation and differentiation of new cells, or on increase in cell size without the need for mitosis and differentiation.

The energy content of the food was reduced by adding non-digestible fibre to standard food without changing its nutrient composition. The resulting high-fibre diet was also coarser than standard diet and required more gizzard grinding activity. As a result of switching from a standard diet to a high-fibre diet, we expected the sizes of gizzard muscle and small intestine to increase. In the small intestine, decreasing quality of the food may be compensated by increasing intestinal length, circumference and surface magnification. With increasing digestive load to the intestine we also expected the muscle layer to thicken (Karasov, 1990, 1996; Martinez del Rio et al., 1994; McWilliams and Karasov, 2001; Hume, 2002). Upregulation of the digestive system incurs energetic costs through protein synthesis, so to maintain a balanced daily energy budget, quail would also need to increase food intake or to fuel flexible responses from adipose tissue.

Possible cellular mechanisms of upregulating the gizzard capacity are hyperplasia, i.e. production of more cells, or hypertrophy, i.e. increased cell size. There is no direct information in birds about the cellular processes underlying an increase in mucosal surface, although it is well known that maintenance of the gut is based on permanent cellular turnover, i.e. a balance between cell proliferation in the intestinal crypts and continued cell loss at the tip of the villi (Altmann, 1972; Johnson and McCormack, 1994; Starck, 1996a,b). In mammals, it has been shown that enlargement of the mucosal absorptive surface is based on increased cell proliferation rates (Williamson and Chir, 1978; Sakata and Engelhardt, 1983; Jacobs and Lupton, 1984; Goodlad et al., 1989; Engelhardt et al., 1989), while elevated levels of apoptosis result in decreased mucosal epithelium (Fleming et al., 1992; Boza et al., 1999; Dunel-Erb et al., 2001). Recent studies (Raab et al., 1998; Mentschel et al., 2001) suggest that in pigs an increase in mucosal absorptive surface may also be based on decreased apical apoptosis. Decreased apoptosis would be energetically cheaper than increased cell proliferation. No information is available for birds, but the similarity of the maintenance system (i.e. cellular turnover) suggests that changes in the mucosal epithelium are caused by a mechanism similar to that in mammals. Liver size has been reported to decline when quail feed on a high-fibre diet (Starck, 1999b). Analysis of liver composition permits an assessment of whether these changes are based on changes in lipid content, i.e. declining energy stores, or on changes in cell numbers, which would imply a changing metabolic capacity of the liver.

## 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 avoid possible fluctuations and elevations of energy requirements due to egg production. Quail were kept in standardized stainless-steel wire boxes (30 cm × 30 cm × 20 cm) according to European Community standards, at a temperature of 22°C with 50% air humidity and a 14 h:10 h light:dark regime.

### *Food*

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. The energy content of the fresh food was 77 kJ $g^{-1}$. Non-digestible fibre (oat beards) was purchased from a commercial mill in Lahr, Germany. The experimental diet contained 40% or 45% non-digestible fibre by mass; consequently, the energy content of the experimental food was reduced by the same amount. During all phases of the study, food and water were offered *ad libitum*.

### *Feeding experiments*

All quail were allowed to acclimatize to the standard food diet for 4 weeks prior to feeding trials, before conducting the following feeding experiments. (1) Control quail were fed the standard diet (control group, $N$=11) and two experimental groups were fed a high-fibre diet containing 40% non-digestible fibre for periods of 2 ($N$=6) and 4 weeks ($N$=6). Quail from this experiment were used to study gross morphological, histological and morphometric changes in response to diet switching. (2) To study the cellular changes associated with changes in gizzard size, 140 quail were acclimatized to the standard diet for 4 weeks. Then, they were divided into a control group remaining on the standard diet and an experimental group (70 birds in each group; beginning of experiment defined as day 0). The experimental group was switched to a diet containing 45% non-digestible fibre. After 14 days, 10 birds from each group were killed, and tissue samples were taken. 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 diet to

experimental diet and back to standard diet was repeated three times, so that in total 7 pairs of groups were taken. (3) To study details of the time course of structural reorganization of the gizzard, 50 quail were fed the standard diet for 4 weeks. At day 0 of the experiment, all quail were switched to a diet containing 45% non-digestible fibre. 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 the previous experiment. Five quail were killed before diet-switching. During the course of the experiment, groups of five quail were killed at intervals of 1–5 days, and tissue samples were taken from the gizzard of these quail. Further details about the experiment are given in Starck (1999b), where data from the same experiment were published. Here, we use tissue material that had not been analyzed previously. An exposure time of 14 days was chosen in all experiments because previous studies had shown that this period allows for full acclimation of the digestive system to the changed diet (Kloss, 1996; Starck, 1999; Dekinga et al., 2001).

### *Dissections and histology*

Animals were killed by cervical dislocation, then immediately dissected macroscopically and organ (gizzard, liver, small intestine) fresh masses determined using a laboratory scale (precision 0.01 g). The organs were preserved in 5% paraformaldehyde in 0.1 mol $l^{-1}$ phosphate buffer, pH 7.4, 4°C, for at least 48 h. Gut length was measured on the preserved material using an electronic slide calliper (precision 0.05 mm), which avoided stretching artefacts that can occur with fresh tissue. To measure the cross-sectional area of gizzard muscles we cut the preserved gizzards longitudinally from the pylorus to the isthmus. An image of the two sides was recorded with a video camera and the image stored on computer. The area of the muscle was then measured using an image analysis program. For histology, tissue samples of the gizzard, small intestine and liver were taken from the preserved material, washed in buffer, dehydrated through a graded ethanol series to 96% ethanol and embedded in hydroxyethyl methacrylate (Historesin). Embedded material was sectioned into short series of 50 sections per sample (section thickness 2 μm), mounted on slides and stained with Methylene-Blue Thionin. Histological sections were studied using a Jenaval research microscope (Zeiss, Jena, Germany) equipped with a video camera and connected to the image-analysis and morphometry system. Microphotographs were taken with a digital camera (Nikon Coolpix 990, Japan). We used SigmaScanPro (Vers. 4.0, Jandel Scientific, SPSS Inc., Chicago, USA) for imaging and morphometry.

### *Morphometry*

We measured 40 sections per tissue sample and took three measurements of muscle layer (tunica muscularis) thickness per section, as a straight line from the inner to the outer margin of the muscle layer. Epithelial surface magnification was measured as the epithelial surface over a baseline defined by the inner circular muscle layer. Measurements were made by tracing the epithelial surface with a cursor on a digitizing tablet and calculation of its total length divided by the length of the baseline, expressed as a dimensionless ratio. There is no standardized quantitative means of assessing the vascularization of a tissue, i.e. the number and density of blood capillaries in a tissue. Injection of microspheres would be one option, but the results depend on blood pressure, blood flow velocity, blood flow volume, flow dynamics, peripheral shunting and branching patterns of larger vessels. We therefore measured the area of tissue in histological sections between the mucosal epithelium and the muscle layer and related it to the sum of the area of the capillaries under that particular epithelium, also expressed as a dimensionless ratio. This procedure does not give absolute values, but a relative measurement of the number, size and density of vessels in a given volume of tissue and, as such, allows us to compare vascularization in the experimental and the control groups.

Fixation of tissue in isotonic and buffered paraformaldehyde, dehydration to 96% ethanol and embedding in methacrylate minimizes embedding artefacts, but the procedure may result in 10% shrinkage of tissue compared to the original size (Böck 1989). Since all tissue samples were treated identically in the present study, however, direct comparisons could be made.

### *Cell proliferation and cellular hypertrophy*

The mitotic index in histological sections is an indication of cell division, the number of mitotic cells per cell population being a direct measurement of cell proliferation. In birds, intestinal cell proliferation of the mucosal epithelium is restricted to the intestinal crypts. We counted mitotic cells in 300 randomly chosen intestinal crypts per gut segment and animal to obtain an estimate of cell proliferation activity in the particular region of the gut of the individual animal.

Cellular hypertrophy leads to increased cell size. In the avian gizzard, smooth muscle cells are arranged in a parallel orientation. We measured the average cross-sectional diameter of smooth muscle cells by counting the number of cells/nuclei crossed by a 1 mm long line perpendicular to the smooth muscle cells. Tissue samples were obtained from five quail per experimental day and from each quail we counted 10 sections. For each bird an individual mean value was calculated before subjecting the values to statistical evaluation to avoid inflation of degrees of freedom.

### *Lipid extraction*

Gizzard and liver were dissected, cleaned from adhering tissue and dried at 55°C to constant mass. Organ fat was extracted in a Soxhlet apparatus using petroleum ether as solvent. For each sample we ran at least 25 cycles to ensure complete extraction of lipids. Lean dry mass (LDM) was calculated as organ fresh mass – (water mass+fat mass).

### *Respirometry*

For the metabolic measurements we used five quail that were adjusted to the standard food diet. Measurements were made

daily (with few exceptions) between 10 h and 16 h with quail that were post-absorptive for 2 h. Respirometry started on day 12 of the experiment. On day 17, the diet was switched to 40% non-digestible fibre, and on day 31, back to the standard diet. The measurements were terminated on day 36. We measured oxygen consumption in an open flow system (FOX Sable Systems, Henderson, NV, USA) at 30°C, i.e. the thermoneutral zone of quail (Feuerbacher, 1981; Kloss, 1996), in the dark for 90 min. The air stream (250 ml $min^{-1}$) was dried (silica gel blue, Roth GmbH, Germany) before entering the metabolic chamber (chamber volume 250 ml). The air stream vented from the metabolic chamber was dried again before entering the oxygen analyzer. We estimated resting metabolic rates (RMR) by taking the lowest value that did not change during a 10 min interval for more than 0.01% $O_2$-concentration. Metabolic data were analyzed with the Sable Systems Inc. software using Withers (1977) equation 3a (assuming a respiratory quotient of 0.83).

### *Statistics*

None of the variables differed from normal distribution. Values are means ± standard deviation (S.D.). We used univariate analysis of covariance (ANCOVA) with body mass as covariate to evaluate the effect of diet on gizzard, intestine and liver morphometric parameters. For analysis of food intake we used repeated-measures (R-M) ANCOVA with body mass as covariate, food composition as inter-subject factor, and day as within-subject factor. We used SPSS Version 11.0 for the analysis of variance (ANOVA). For the analysis of metabolic measurements we first calculated a linear regression of oxygen consumption against time over the whole course of the experiments to assess long-term trends. Regressions were calculated for all birds together and then grouped by individual quail to assess individual reactions. As there was no indication of any consistent trend over time we analyzed the three experimental periods (standard diet, experimental diet or reassumed standard diet) separately by repeated-measures restricted maximum likelihood estimation (REML) with body mass as covariate. These analyses were performed using Genstat, 5th edition.

## Results

### *Body mass and food intake*

During experiment 1, mean body mass of control birds was 148.7±0.5 g (*N*=6) over the experimental period, whereas body mass of experimental birds declined from 148.5±5.3 g (*N*=6) on day 1 of the experiment to 142.2±6.3 g on day 6. Thereafter, body mass resumed values that were not different from control (Fig. 1). Due to high interindividual variability, the changes in body mass were not statistically significant between days of the experiment.

During the acclimation period, quail ingested on average 14.2±2.8 g (*N*=12) standard food per day. During experiment 1, control birds ate on average 14.8±1.0 g (*N*=6) food each day, although they ate less food on the first day compared to the last 5 days (Fig. 2). Diet composition had a highly significant effect on food intake (R-M ANCOVA with body mass as covariate; d.f.=1,9; $F$=53.851; $P$<0.001; body mass was not significant as a covariate $F$=3.758, $P$=0.85; Fig. 2). After switching to the high-fibre diet, experimental quail reduced their food intake to less than 50% of the control amount (6.3±1.2 g, *N*=6). After 3 days on the high-fibre diet, the quails resumed food intake rates

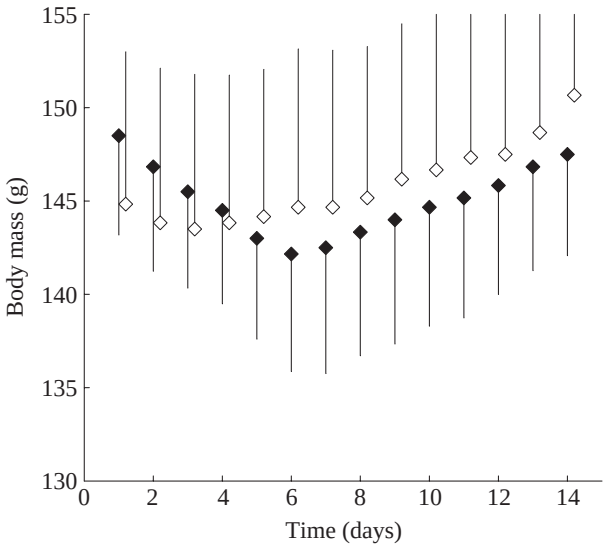


Fig. 1. Body mass changes in response to diet-switching. On day 0, food was switched from standard to a high-fibre diet (40% fibre) in the experimental group (black symbols). Animals in the control group (white symbols) continued to feed on standard diet during the entire period. Values are means ± S.D., *N*=6 for each group.

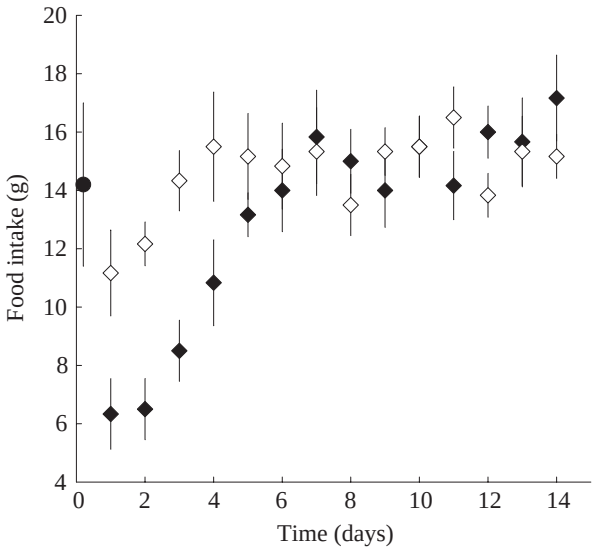


Fig. 2. Food intake in control (white symbols) and experimental group (black symbols) when switched from standard diet to high-fibre diet (40% fibre). Values are means ± S.D., *N*=6 for each group. The black circle indicates mean standard food intake of all 12 birds before the experiment started.

Table 1. *Effect of food composition on gizzard and intestine cross morphometry (univariate ANCOVA with body mass as covariate)*

| | Standard food | | Duration of feeding high-fibre diet | | | |
|---|---|---|---|---|---|---|
| | | *N* | 2 weeks | *N* | 4 weeks | *N* |
| Gizzard fresh mass (g)*** | 2.61±0.36[a] | 11 | 4.77±0.50[b] | 6 | 6.01±0.5[c] | 5 |
| Gizzard length (mm)*** | 21.73±2.41[a] | 11 | 30.32±3.32[b] | 6 | 37.60±2.07[c] | 5 |
| Gizzard muscle ($mm^2$)** | 128.70±19.85[a] | 6 | 180.53±42.15[b] | 6 | – | 5 |
| Gizzard LDF (g)* | 0.73±0.07[a] | 11 | 0.92±0.09[b] | 6 | 1.29±0.14[c] | 5 |
| Gizzard lipid%* | 12.41±1.23[a] | 11 | 9.56±0.78[b] | 6 | 11.15±0.94[b] | 5 |
| Duodenum length (mm)*** | 95.00±5.80[a] | 11 | 109.17±6.46[b] | 6 | 122.60±4.51[b] | 5 |
| Jejunoileum length (mm)*** | 279.00±16.1[a] | 11 | 313.17±10.67[b] | 6 | 342.00±9.54[b] | 5 |
| Rectum length (mm)*** | 28.64±2.80[a] | 11 | 35.67±1.75[b] | 6 | 39.60±2.97[b] | 5 |

LDF, lean dry fraction; *N* = number of birds.

Body mass was not significant as a covariate.

Gizzard fresh mass, d.f.=1,19; $F$=63.21; $P$<<0.001; length d.f.=1,19; $F$=41.23; $P$<<0.001; muscle, d.f.=1,19; $F$=11.40; $P$=0.008; LDF, d.f.=1,19; $F$=15.55; $P$=0.001; lipid%, d.f.=1,19; $F$=9.67; $P$=0.006.

*Post-hoc* test (univariate ANOVA, REGWQ) for differences among means; means labelled with different letters are significantly different; ***$P$=0.001; **$P$=0.005; *$P$=0.01.

similar to the rate before the diet switch. Resumption of the intake rate coincided with stabilization and regain of body mass on day 6 of experiment.

### *Gizzard size changes*

Gizzard fresh mass in quail fed the high-fibre diet for 14 days was 182% of gizzard mass in the control group. Gizzard length and cross-sectional area of gizzard muscles of experimental birds were 140% of those of controls. Lean dry fraction (LDF) of the gizzard was 126% of the control value, indicating that a major portion of the size change was based on increased protein fraction, while water and lipids did not contribute much to the fresh mass increase (Table 1). After 4 weeks feeding on the high-fibre diet, gizzard fresh mass was 230% and gizzard length was 173% of the size in control birds. The LDF reached 263% of control values. ANCOVA with body mass as covariate showed that diet composition was a significant factor for all measured parameters. In no case was body mass significant as a covariate. *Post-hoc* comparisons among means showed highly significant differences between control and experimental groups for all measured parameters (Table 1). Gizzard fresh mass and gizzard length increased with exposure time, i.e. were significantly larger after 4 weeks than after 2 weeks exposure time. The other measurements were not affected by the length of the feeding trial.

### *Gizzard histology*

The muscles of the avian gizzard consist of layers of smooth muscle cells separated by thin sheets of connective tissue (Fig. 3) resulting in an ‘onion-structure’ of the gizzard muscle. Within each muscle layer, the smooth muscle cells show the typical elongated spindle shape with the peripheral compartment of contractile fibres and the perinuclear cytoplasm. The nucleus is cigar-shaped, about five times as long as wide.

We analysed tissue material from experiment 2, in which quail were repeatedly switched at biweekly intervals between standard and high-fibre diet. The control group was kept in parallel without any changes of diet. Animals from both groups were measured at 2-week intervals. We compared the effects of the following factors on number of nuclei in the gizzard muscle: the factor ‘repeat’ tested for differences between the repeated diet switches, the factor ‘group’ tested for overall differences between experimental and control group, and the factor ‘food’ tested for differences between high fibre and standard diet. The effect of diet change was assessed by the interaction term between ‘food’ and ‘group’. No interaction would indicate that the groups did not differ, despite the differences in diet. Significant differences would indicate an effect of diet on the number of nuclei. We discarded body mass as a covariate from the analysis, because it was not significant in any of the previous analyses of gizzard size changes. Repeat was a significant factor (univariate ANOVA, d.f.=3,47; $F$=3.66; $P$=0.019), thus food composition and the number of repeated diet switches affected the structural response. It was not an increased response (upregulation) that was stronger with repeated diet switches but the decreased response (downregulation), which did not always return to control values (Fig. 4). This observation was consistent with gizzard mass not returning to its original value during downregulation. After switching from standard diet to high-fibre diet, the number of nuclei $mm^{-1}$ declined significantly, indicating an increase in smooth muscle cross section (Fig. 4A). The experimental and control groups differed in their overall number of nuclei $mm^{-1}$. Gizzards of control birds had significantly more nuclei $mm^{-1}$ than the experimental birds. During the entire experimental period, the control group was unchanged. Overall, the effect of food was significant, with higher numbers of nuclei in birds fed the standard diet (d.f.=1,47; $F$=15.08; $P$<0.001). However, the interaction between food and group was highly significant (d.f.=1,47, $F$=22.79; $P$<0.001), indicating a massive

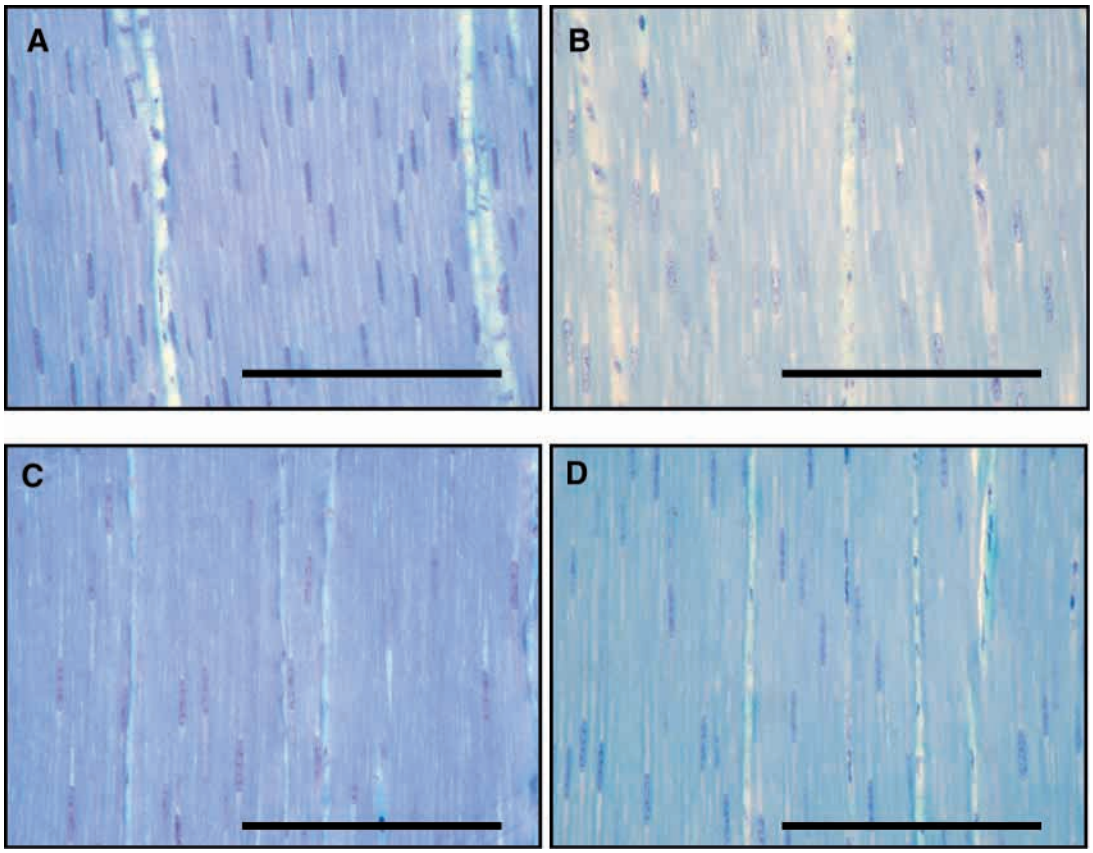


Fig. 3. Histology of quail gizzard muscle. (A) Control, (B) 4 days after switching to the high-fibre diet (40% fibre), (C) 10 days after switching to the high-fibre diet, (D) 4 days after switching from the high-fibre diet back to standard food. Scale bars, 100 μm.

difference between control and experimental birds. In the experimental birds, switching back to standard diet resulted in an increase of nuclei $mm^{-1}$, i.e. a decrease of muscle cell cross section (Figs 3A–C, 4). Changes in both directions could be elicited repeatedly. At a more detailed level, we observed a decline in number of nuclei $mm^{-1}$ within 1 day of switching to the high-fibre diet (Fig. 4B). 5 days after diet switching, the number of nuclei $mm^{-1}$ was at 50% of the level of the control group, and it remained this low as long as the high-fibre diet was offered. When the food was replaced by the standard diet, the number of nuclei $mm^{-1}$ increased, indicating a decrease in cell size. Subsequent values were not significantly different from each other, but differences were significant when longer intervals were compared (univariate ANOVA with food and day as factors, *post-hoc* REGWQ test at $P$=0.01), e.g. comparisons between days 1 and 4 after diet switching in either direction rendered significantly different values (Fig. 4B). Despite the changes in gizzard size, we did not observe any mitotic structures in satellite cells. Thus, cell proliferation can be excluded as a possible source of organ size increase.

### *Gut morphometry*

The duodenum of quail fed the high-fibre diet for 14 and 28 days was 115% and 130% longer, respectively, than the length in control birds (Table 1). The length of the jejunoileum was 112% of the control length after 2 weeks and 123% after 4 week, and the length of the rectum had increased after 2 weeks (125%) and 4 weeks (138%) of experimental diet. All reported length differences were statistically highly significant between control and experimental groups (ANCOVA with body mass as covariate; see Table 1 for statistics). However, gut lengths of quail fed the high-fibre diet for 2 and 4 weeks were not significantly different (ANOVA, *post-hoc* REGWQ test; Table 1), so for histological examinations we used only tissue material from quail fed for 2 weeks on the high-fibre diet. The circumference of the gut and the thickness of the muscle layer (Tunica muscularis) changed in response to diet composition. Both measurements in quail fed the high-fibre diet were significantly higher, except for the thickness of muscle layer in the rectum, than in control birds (ANCOVA with body mass as covariate; see Table 2 for statistics). The surface area of the small intestine of quail fed the high-fibre diet was enlarged in all three segments of the intestine (Table 2). Enlargement of the duodenal surface of quail fed the 40% non-digestible diet was 130% of the control, and the jejunoileum and rectum were 151% and 149% of control levels, respectively (Table 2).

When compared to control birds, the number of mitotic cells per intestinal crypt of quail fed the high-fibre diet was 140% in the duodenum, 155% in the jejunoileum and 117% in the rectum. Differences between controls and experimental group were significant for all parts of the gut (Table 2).

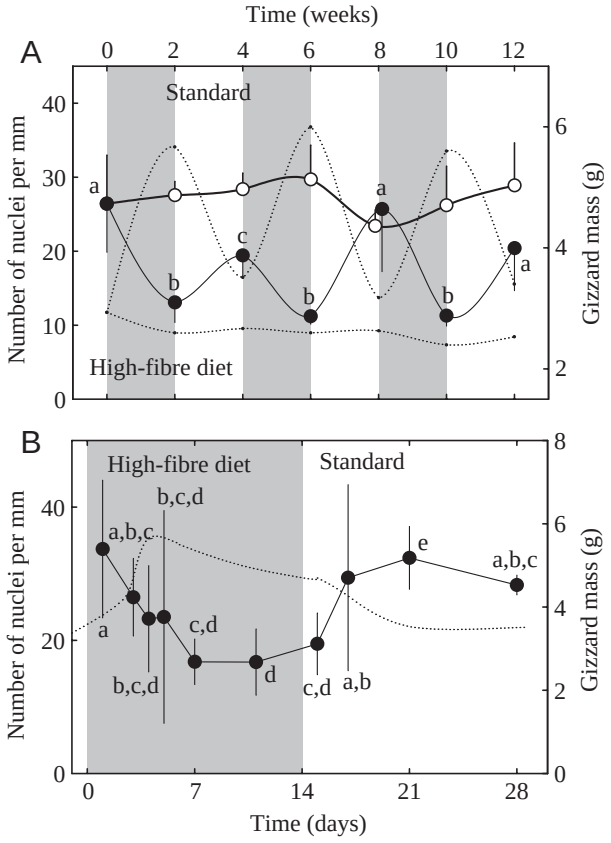


Fig. 4. Hypertrophy of smooth muscle cells in response to feeding a high-fibre diet. (A) Number of nuclei $mm^{-1}$ change in response to repeated switches from standard food (white areas) to the high-fibre diet (45% fibre, tinted area). Values are means ± S.D. ($N$=5); black symbols, experimental animals; white symbols, control. (B) Time course of hypertrophy of smooth muscle cells in response to the high-fibre diet. In A and B, the dotted lines refer to associated gizzard size changes (right axis) of experimental and control groups as reported in Starck (1999b). Mean values denoted by different letters differ significantly according to the REGWQ-test, $P$<0.001.

### *Vascularization*

The vascularization of duodenal and jejunoileum tissue in quail fed the high-fibre diet was 205% and 214% of control birds, respectively. However, the effect of food composition was only marginally significant (ANCOVA with body mass as covariate; Table 2). An elevated level of vascularization in the rectum of experimental birds (137%) was not significantly different from the control.

### *Liver mass, histology and organ composition*

Liver fresh mass decreased to 78% and 79% of control values in quail fed the high-fibre diet for 2 and 4 weeks, respectively. The differences between experimental birds and control were significant (ANCOVA with body mass as covariate; Table 3). The LDF of the liver did not change in response to food composition; a slight decline in LDF during a 2 week exposure to high fibre was not significant. Lipid content was significantly lower in experimental animals than in control birds. Thus, differences in liver fresh mass were based on changes in the lipid (and water) components of liver but not on the protein component. Change in cell numbers can be excluded as a cause of change in organ size.

Table 2. *Effect of food composition on gut morphometry (univariate ANCOVA with body mass as covariate)*

| | Standard diet | High-fibre diet |
|---|---|---|
| Circumference | | |
| Duodenum (mm)** | 8.99±0.18$^a$ | 10.50±0.32$^b$ |
| Jejunoileum (mm)** | 6.97±0.11$^a$ | 8.06±0.30$^b$ |
| Rectum (mm)** | 7.60±0.25$^a$ | 8.78±0.48$^b$ |
| Thickness of muscularis | | |
| Duodenum (mm)*** | 0.05±0.01$^a$ | 0.09±0.01$^b$ |
| Jejunoileum (mm)*** | 0.05±0.01$^a$ | 0.07±0.01$^b$ |
| Rectum (mm)* | 0.17±0.05$^a$ | 0.26±0.08$^a$ |
| Surface magnification | | |
| Duodenum*** | 8.21±0.72$^a$ | 10.70±1.12$^b$ |
| Jejunoileum** | 4.97±0.42$^a$ | 7.50±1.39$^b$ |
| Rectum** | 4.28±0.57$^a$ | 6.36±1.15$^b$ |
| Number of mitosis figures | | |
| Duodenum*** | 6.90±0.63$^a$ | 9.66±0.47$^b$ |
| Jejunoileum*** | 4.88±1.06$^a$ | 7.57±0.73$^b$ |
| Rectum* | 5.43±0.57$^a$ | 6.36±0.83$^b$ |
| Vascularization | | |
| Duodenum* | 0.20±0.07$^a$ | 0.41±0.61$^b$ |
| Jejunoileum* | 0.14±0.01$^a$ | 0.30±0.09$^b$ |
| Rectum$^{NS}$ | 0.17±0.02$^a$ | 0.23±0.06$^a$ |

$N$=6 for all measurements.

Body mass was not significant as a covariate.

Circumference duodenum, d.f.=1,9; $F$=66.49; $P$<0.001; circumference ileum, d.f.=1,9; $F$=42.57; $P$=<0.001; circumference rectum, d.f.=1,9; $F$=12,27; $P$=0.007.

Thickness of muscularis duodenum, d.f.=1,9; $F$=15.79; $P$=0.003; thickness of muscularis jejunoileum, d.f.=1,9; $F$=18.79; $P$=0.002; thickness of muscularis rectum, d.f.=1,9; $F$=7.34; $P$=0.024.

Surface increase of duodenum, d.f.=1,9; $F$=15.2; $P$=0.003; surface increase of ileum, d.f.=9; $F$=17.89; $P$=0.002; surface increase of rectum, d.f.=1,9; $F$=15.16; $P$=0.004.

Mitoses in duodenum, d.f.=1.9; $F$=190.4; $P$<0.001; mitoses in ileum, d.f.=1.9; $F$=15.8; $P$=0.003; mitoses in rectum, d.f.=1.9; $F$=1.8; $P$=0.212.

Vascularization of duodenum, d.f.=1,9; $F$=4.19; $P$=0.07; vascularization of ileum, d.f.=1,9; $F$=5.37; $P$=0.046; vascularization of rectum, d.f.=1.9; $F$=1.84; $P$=0.21.

*Post-hoc* test (univariate ANOVA, REGWQ) for differences among means; means labelled with different letters are significantly different; ***$P$=0.001; **$P$=0.005; *$P$=0.01.

### *Metabolic rates*

We measured oxygen consumption in five quail over a period of 24 days. Measurements started 5 days prior to switching from standard food to the high-fibre diet. Daily measurements continued during the 14 days of feeding the high-fibre diet, and for another 5 days after switching back to standard food (Fig. 5).

Table 3. *Effect of food composition on liver morphometry (ANCOVA with body mass as covariate)*

| | Standard food | | Duration of feeding high-fibre diet | | | |
|---|---|---|---|---|---|---|
| | | *N* | 2 weeks | *N* | 4 weeks | *N* |
| Liver fresh mass (g)*** | 3.31±0.29[a] | 11 | 2.57±0.13[b] | 6 | 2.61±0.13[b] | 5 |
| Lean dry fraction (g)*** | 1.00±0.18[a] | 11 | 0.86±0.08[a] | 6 | 1.09±0.03[a] | 5 |
| Lipid%*** | 14.26±1.31[a] | 11 | 9.95±1.27[b] | 6 | 11.74±0.82[a,b] | 5 |

Body mass was not significant as a covariate.

Liver fresh mass, d.f.=1,19; $F$=38.11; $P$<0.001; liver lean dry fraction, d.f.=1,19; $F$=1.26; $P$=0.276; lipid contents%, d.f.=1,19; $F$=24.4; $P$<0.001.

*Post-hoc* test (univariate ANOVA, REGWQ) for differences among means; means labelled with different letters are significantly different; ***$P$=0.001.

We observed a decline in metabolic rate just before diet switching that was significant when analyzed by repeated measures REML of oxygen consumption over the 4 days of pre-treatment with body mass as covariate: effect of day, Wald-statistics = 5.29 on 3 d.f., $P$=0.001; covariate body mass, Wald-statistics = 3.02 on 1 d.f., $P$=0.082). In addition, individual quail clearly differed from each other (differences between quail, Wald-statistics = 13.15 on 4 d.f., $P$<0.001). This metabolic decline was associated with the birds acclimating to the experimental conditions. There were no significant changes in resting metabolic rate (RMR) after birds switched diets (oxygen consumption over the 14 days of food treatment: effect of day, Wald-statistics = 1.19 on 9 d.f.; $P$=0.298; covariate body mass, Wald-statistics = 0.87 on d.f. 1; $P$=0.351; oxygen consumption over the 6 days after shifting back to the standard diet: effect of day, Wald-statistics = 1.54 on 5 d.f.; $P$=0.173; covariate body mass, Wald-statistics = 0.69 on 1 d.f.; $P$=0.406). In contrast, the differences between individual quail remained stable (oxygen consumption over the 14 days of food treatment: effect of quail, Wald-statistics = 15.51 on 4 d.f.; $P$<0.001; over the 6 days after shifting back to standard diet: effect of quail, Wald-statistics = 5.03 on 4 d.f.; $P$<0.001). The mean oxygen consumption of the five quail among the three experimental intervals showed no effect of experimental interval (pre-treatment, treatment, post-treatment) on oxygen consumption but, again, clearcut differences between individual quail (effect of experimental interval, Wald-statistics = 0.65 on 2 d.f.; $P$=0.521; effect of quail, Wald-statistics = 2.58 on 4 d.f.; $P$=0.035; effect of covariate body mass, Wald-statistics = 0.51 on 1 d.f.; $P$=0.476). This analysis showed clearly that oxygen consumption differed between quail and was affected by initial acclimation but not by the change of diet. Thus, once the animals had successfully habituated to the experimental conditions, the resting metabolic rates were constant throughout the experiment.

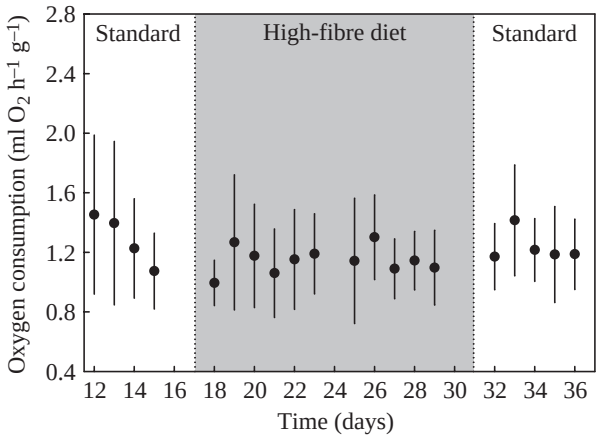


Fig. 5. Oxygen consumption of quail fed standard food (white areas) and high-fibre diet (titnted areas). Values are means ± S.D. of 5 adult male quail.

## Discussion

Quail fed the high-fibre diet reduced food intake immediately after the beginning of experiments, but resumed normal rates of food intake 6 days later. This reduction of intake rate follows the same patterns reported in Starck (1999b). Reduced food intake rates were associated with increased gizzard size, and increasing intestine size. Resting metabolic rates were not affected by switching diet to a low energy food or by up- and downregulation of the gastrointestinal capacity, in contrast to our predictions that feeding on a low energy diet would result in increasing food intake rates, or lowered resting metabolic rates. Obviously, quail metabolized internal energy stores to increase organ size and kept RMR constant. Liver lipid content decreased after diet switching, indicating that lipids stored in the liver were mobilized as fuel for the upregulation of the digestive system. As a future research perspective it would be interesting to find out if this response is condition dependent, i.e. if the response depends on the amount of adipose tissue. We did not measure body composition in the present study, but it was obvious from dissections that quail lost adipose tissue when fed a diet containing 40% non-digestible diet. A straightforward interpretation is that quail fuel the flexible responses of their gastrointestinal tract by mobilizing lipid stores from liver and adipose tissue, but keeping RMR constant.

Normal or elevated intake rates were re-established after 6 days, i.e. when organ size had been enlarged to accommodate the functional demands of the changed diet composition. The amount and period of decline of food

intake and the later increase of intake rates were similar to those reported by Starck (1999b) for a diet containing 45% non-digestible fibre. Because of the reduced intake, it is not the load to the gastrointestinal tract that causes enlargement of the gizzard but the quality of the food (reduced energy content, increased coarseness) that triggers the observed phenotypic changes of the gastrointestinal tract. Increased food intake only follows after the digestive tract has been structurally reorganized and can accommodate increased loads of digesta.

### *Flexible changes of the gastrointestinal tract*

#### *Gizzard*

The observed size changes of the muscular stomach support our earlier findings (Starck, 1999b). Mean gizzard mass was 4.8±0.5 g after 2 weeks experimental exposure, which is within the prediction limits of 5.3±0.9 g (Starck, 1999b). Interestingly, gizzard size continued to increase over a period of 4 weeks, reaching a maximum size of 6.01±0.5 g. This adds a new perspective to our earlier report that the maximum possible gizzard size in Japanese quail was 6 g when fed a diet containing 45% non-digestible fibre for 2 weeks (Starck, 1999b). In the present study, we found that an extended feeding period resulted in a further increase in gizzard size, even when the original fibre-load was not strong enough to elicit the maximum response. We conclude that the flexible response of gizzard size is determined by a combination of diet quality and exposure time. Extended exposure time causes continued organ growth until the upper ceiling is reached.

Increase in gizzard mass was associated with an increase in lean dry fraction (LDF), while lipid proportions did not change. Changes in gizzard size were the result of changes in smooth muscle cell size, i.e. hypertrophy and cellular atrophy during enlargement and reduction, respectively. We did not detect any increase in mitotic index in several hundred histological slides examined. Therefore, we conclude that the increased gizzard size is solely due to hypertrophy of smooth muscle cells. Hypertrophy and cellular atrophy could be elicited repeatedly and were synchronized with changes in organ size.

#### *Intestines*

The high-fibre diet had a decreased energy content and increased coarseness compared to standard food. The digestive modulation model (Karasov, 1990; Martinez del Rio et al., 1994) predicts an increase of intestine size (length, circumference, surface magnification) in response to decreasing food quality. Taking the different segments of the gastro-intestinal tract as cylinders, we calculated a volume of 1821 $mm^3$ for quail fed standard food, and 2795.6 $mm^3$ for experimental animals, which is in agreement with the digestive modulation model. The associated increase in vascularization indicates that 'downstream' transport mechanisms respond in concert with the mucosal epithelium. Increased muscle layer thickness is certainly related to increased bulk.

The elevated levels of cell proliferation in the intestinal crypts after 2 weeks on the high-fibre diet show that enlargement of the mucosal epithelium is based on proliferation of new cells. In birds, we do not observe apoptosis at the tip of the villi. The extrusion rate of epithelial cells cannot be measured directly, but we must expect that cell extrusion will also be elevated because it balances cell proliferation as soon as the upregulated state is reached. To our knowledge, this is the first study to examine changes in mitotic index in bird intestine in response to diet switching, so our conclusions are restricted to quail. For mammals, increased cell proliferation (rats and sheep; Sakata and Engelhardt, 1983; Engelhardt et al., 1989) as well as a change in apoptosis rate (pigs; Raab et al., 1998; Mentschel et al., 2001) were reported to be mechanisms that respond to diet switching and change the mucosal surface area. In mice/rats, increasing the fibre content of food induced mitosis in the small intestine (Sakata and Engelhardt, 1983; Jacobs and Lupton, 1984; Engelhardt et al., 1989; Goodland et al., 1989; Fleming et al., 1992). We observed a similar pattern in quail but the underlying regulatory mechanisms remain unresolved.

#### *Liver*

Liver fresh mass declined during the experiment, but this was exclusively based on declining lipid content of the liver. LDF of the liver was constant during the experiment, indicating that no cell proliferation had occurred. This makes immediate sense because the liver is an intermediate store for lipids, which were obviously metabolized to fuel the upregulation of the gastrointestinal system. Interestingly, the lipid content of the liver of quail fed the high-fibre diet for 4 weeks was intermediate between the levels in controls and in quails fed the high-fibre diet for only 2 weeks. The 4-week group could not be distinguished statistically from the others. A possible interpretation is that a 4 week period allows time for better overall acclimation of the digestive system to the high-fibre diet and for reestablishment of a nutritional status equivalent to that on the standard diet.

### *Metabolic rates*

Diet switching did not significantly affect RMR and mean oxygen consumption was 1.19±0.31 ml $O_2$ $h^{-1}$ $g^{-1}$. Our measurements are the same as the mean values reported by Kloss (1996), who measured RMR of adult (male) quail in the thermoneutral zone as 1.18±0.22 ml $O_2$ $h^{-1}$ $g^{-1}$. She also compared quail fed different diets and showed that RMR was constant, although digestive efficiency as well as morphological parameters of the digestive tract changed. The constancy of RMR is an interesting contrast to the flexible metabolic rates of growing quail (Schew, 1995; Schew and Ricklefs, 1998). When young quail were temporarily malnourished their RMR decreased by 40%. The young of songbirds also lowered their RMR when subjected to periods of fasting or malnutrition (Konarzewski and Starck, 2000; Brzek and Konarzewski, 2001). There are two possible interpretations: (1) at some time during ontogeny quail lose

the flexibility of RMR, or (2) quail do not lose their flexible RMR, but our experimental conditions did not meet the requirements necessary to elicit a reduced RMR in adults. If flexible responses to changes in diet are condition dependent, we may hypothesize that well-fed quail simply use their adipose tissue stores to fuel the flexible responses of their gastrointestinal tract. That way, they tolerate a temporarily negative energy budget for a few days but they keep their RMR constant. If, however, quail were in a poor condition, they might have no fuel for the flexible responses and might have to reduce energy expenditure to other organ systems, thus reducing their RMR. Both ideas need to be tested before final conclusions can be drawn. Condition dependency of metabolic response is indirectly supported by a study of emperor *Aptenodytes forsteri* and king penguins *A. patagonicus*, which reduce RMR only after extended fasting periods before entering starvation, characterized by mobilization of body protein (Groscolas and Cherel, 1992). Also, Klaassen and Biebach (1994) showed that garden warblers *Sylvia borin* reduce RMR during starvation.

### *Overall conclusions about organ size flexibility*

The data presented in this study show that different processes account for the organ size changes of gizzard, intestine and liver. In the gizzard, size changes are based on up- (cellular hypertrophy) and downregulation (cellular hypotrophy) of cell size. In contrast, size changes of the mucosal epithelium of the intestine are based on changes in the balance of cell proliferation and cell loss, and involve increased intestinal crypt cell proliferation. Finally, liver size changes are based on changes in organ lipid contents. The costs of flexibility of the digestive system are paid by metabolizing body fuel, while the RMR stays constant. The condition dependency of the responses still needs to be tested.

The limited amount of available data restricts a comparative perspective. Studies on epithelial renewal of small intestine in birds and mammals showed that a continuous proliferation of cells in the intestinal crypts not only drives the renewal of the epithelium, but also allows for fast and reversible changes of mucosal epithelial surface (Starck, 1996a,b, 1999a,b). Our data as well as published data on mammals indicate that elevated levels of cell proliferation are important in increasing mucosal epithelium surface, but rates of cell loss have not yet been examined.

Note that ectotherm sauropsids also show a considerable up- and downregulation of their gastrointestinal system in response to feeding, but that the underlying tissue mechanisms are different. In snakes (Starck and Beese, 2001, 2002) and crocodiles (Starck et al., 2002), the pseudostratification of the mucosal epithelium allows for rapid, reversible and repeated up- and downregulation of the small intestine capacity. The tissue mechanism of upregulation in ectotherm sauropsids is energetically cheap. The different mechanisms observed in ectotherm sauropsids, birds and mammals suggest independent evolutionary origins of the flexibility of the gastrointestinal system in each of the three taxa.

We thank Sybille Koch for skilful help in the laboratory. We gratefully acknowledge statistical advice and comments on an earlier version of this paper by Barbara Helm. Two anonymous reviewers provided helpful comments and suggestions to improve the paper. The research was supported by a grant from the German Research Council (STA 345/4-2) to J.M.S. and a PhD fellowship through the channel system of the Egyptian Ministry of Education and Science to G.H.A.R.

## References

**Altmann, G. G.** (1972). Influence of starvation and refeeding on mucosal size and epithelial renewal in the rat small intestine. *Am. J. Anat.* **133**, 391-400.

**Böck, P.** (1989). *Romeis' Mikroskopische Technik*. 17th edition. München: Urban and Schwarzenberg.

**Boza, J. J., Moënnoz, D., Vuichoud, J., Jarret, A. R., Gaudard-de-Weck, D., Fritsché, R., Donnet, A., Schiffrin, E. J., Peruissaeu, G. and Ballèvre, O.** (1999). Food deprivation and refeeding influences growth, nutrient retention and functional recovery of rats. *J. Nutr.* **129**, 1340-1346.

**Brzek, P. and Konarzewski, M.** (2001). Effect of food shortage on the physiology and competitive abilities of sand martin (*Riparia riparia*) nestlings. *J. Exp. Biol.* **204**, 3065-3074.

**Dekinga, A., Dietz, M. W., Koolhaas, A. and Piersma, T.** (2001). Time course and reversibility of changes in the gizzard of red knots alternately eating hard and soft food. *J. Exp. Biol.* **20**, 2167-2173.

**Diamond, J. M.** (1998). Evolution of biological safety factors: a cost/benefit analysis. In *Principles of Animal Design* (ed. E. R. Weibel, C. R Taylor and L. Bolis), pp. 21-27. Cambridge: Cambridge University Press.

**Diamond, J. M. and Hammond, K. A.** (1992). The matches, achieved by natural selection, between biological capacities and their natural loads. *Experientia* **48**, 551-557.

**Dunel-Erb, S., Chevalier, C., Laurent, P., Bach, A., Decrock, F. and le Maho, Y.** (2001). Restoration of the jejunal mucosa in rats refed after prolonged fasting. *Comp. Biochem. Physiol.* **129A**, 933-947.

**Engelhardt, von W., Rönnau, K., Rechkemmer, G. and Sakata, T.** (1989). Absorption of short-chain fatty acids and their role in the hindgut of monogastric animals. *Anim. Feed Sci. Technol.* **23**, 43-53.

**Feuerbacher, I.** (1981). Der Einfluß von Testosteron auf die Regulation der Körpertemperatur und den Energiestoffwechsel bei der Wachtel (*Coturnix coturnix japonica*). *PhD Dissertation*, University of Tübingen.

**Fleming, S. E., Fitch, M. D. and de Vries, S**. (1992). The influence of dietary fibre on proliferation of intestinal mucosal cells in miniature swine may not be mediated primarily by fermentation. *J. Nutr.* **122**, 906-916.

**Garland, T., Jr** (1998). Conceptual and methodological issues in testing the predictions of symmorphosis. In *Principles of Animal Design* (ed. E. R. Weibel, C. R. Taylor and L. Bolis), pp. 40-47. Cambridge: Cambridge University Press.

**Goodland, R. A., Ratcliffe, B., Fordham, J. P. and Wright, N. A.** (1989). Does dietary fibre stimulate intestinal epithelial cell proliferation in germ free rats? *Gut* **30**, 820-825.

**Groscolas, R. and Cherel, Y.** (1992). How to molt while fasting in the cold – the metabolic and hormonal adaptations of emperor and king penguins. *Ornis Scand.* **23**, 328-334.

**Hammond, K. A.** (1998). The matches between load and capacity during lactation: where is the limit to energy expenditure? In *Principles of Animal Design* (ed. E. R. Weibel, C. R. Taylor and L. Bolis), pp. 205-211. Cambridge: Cambridge University Press.

**Hammond, K. A., Konarzewski, M., Torres, R. M. and Diamond, J.** (1994). Metabolic ceilings under a combination of peak energy demands. *Physiol. Zool.* **67**, 1476-1506

**Hammond, K. A., Lam, M., Lloyd, K. C. C. and Diamond, J.** (1996). Simultaneous manipulation of intestinal capacities and nutrient loads in mice. *Am. J. Physiol.* **271**, G969-G979.

**Hume, I. D.** (1998). Optimization in design of the digestive system. In *Principles of Animal Design* (ed. E. R. Weibel, C. R. Taylor and L. Bolis), pp. 212-219. Cambridge: Cambridge University Press.

**Hume, I. D.** (2002). Digestive strategies of mammals. *Acta Zool. Sinica* **48**, 1-19.

**Hume, I. D, Geiglböck, C., Ruf, T., Frey-Roos, F., Bruns, U. and Arnold, W.** (2002). Seasonal changes in morphology and function of the

gastrointestinal tract of free-living alpine marmots (*Marmota marmota*). *J. Comp. Physiol. B* **172**, 197-207.

**Imondi, A. R. and Bird, F. H.** (1966). The turnover of intestinal epithelium in the chick. *Poult. Sci.* **45**, 142-147.

**Jacobs, L. R. and Lupton, J. R.** (1984). Effect of dietary fibres on rat large bowel mucosal growth and cell proliferation. *Am. J. Physiol.* **246**, G378-G385.

**Johnson, L. R. and McCormack, S. A.** (1994). Regulation of gastrointestinal mucosal growth. In *Physiology of the Gastrointestinal Tract*, 3rd edition (ed. L. R. Johnson), pp. 611-641. New York: Raven Press.

**Karasov, W. H.** (1990). Digestion in birds: chemical and physiological determinants and ecological implications. *Studies Avian Biol.* **13**, 391-415.

**Karasov, W. H.** (1996). Digestive plasticity in avian energetics and feeding ecology. In *Avian Energetics and Nutritional Ecology* (ed. C. Carey), pp. 61-84. New York: Chapman and Hall.

**Klaassen, M. and Biebach, H.** (1994). Energetics of fattening and starvation in the long-distance migratory garden warbler, *Sylvia borin*, during the migratory phase. *J. Comp. Physiol. B* **164**, 362-371.

**Kloss, E.** (1996). Morphometrische Untersuchungen am Intestinaltrakt von Vögeln. Morphologische und physiologische Reaktionen auf veränderte Nahrung. PhD dissertation, University of Tübingen, Germany.

**Konarzewski, M. and Diamond, J.** (1994). Peak sustained metabolic rate and its individual variation in cold-stressed mice. *Physiol. Zool.* **67,** 1186-1212.

**Konarzewski, M. and Starck, J. M.** (2000). Developmental plasticity of the gastrointestinal tract in song thrush nestlings. *Physiol. Biochem. Zool.* **73**, 416-427.

**Lilja, C.** (1987). Mitotic activity of duodenal crypts in the young fieldfare, *Turdus pilaris*. *Acta Physiol. Scand.* **131**, 163-164.

**Lilja, C. and Amneus, H.** (1987). Postnatal growth of the liver in the young fieldfare (*Turdus pilaris*). *Growth* **51**, 181-188.

**Martinez del Rio, C., Cork, S. J. and Karasov, W. H.** (1994). Modelling gut function: an introduction. In *The Digestive System in Mammals* (ed. D. J. Chivers and P. Langer), pp. 25-53. Cambridge: Cambridge University Press.

**McNab, B. K.** (2002). *The Physiological Ecology of Vertebrates. A View from Energetics*. Cornell: Cornell University Press.

**McWilliams, S. R. and Karasov, W. H.** (2001). Phenotypic flexibility in the digestive system structure and function in migratory birds and its ecological significance. *Comp. Biochem. Physiol.* **128A**, 579-593.

**Mentschel, J., Leiser, R., Mülling, C., Pfarrer, C. and Claus, R.** (2001). Butyric acid stimulates rumen mucosa development in the calf mainly by a depression of apoptosis. *Arch. Anim. Nutr.* **55**, 85-102.

**Piersma, T.** (2002). Energetic bottlenecks and other design constrains in avian annual cycles. *Integ. Comp. Biol.* **42**, 51-67.

**Piersma, T. and Drent, J.** (2003). Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.* (in press).

**Piersma, T. and Lindström, A.** (1997). Rapid reversible changes in organ size as a component of adaptive behavior. *Trends Ecol. Evol.* **12**, 134-138.

**Raab, S., Leiser, R., Kemmer, H. and Claus, R.** (1998). Effects of energy and purines in the diet on proliferation, differentiation, and apoptosis in the small intestine of the pig. *Metabolism* **47**, 1105-1111.

**Sakata, T. and Engelhardt, von W.** (1983). Stimulatory effect of short chain fatty acids on the epithelial cell proliferation in the rat large intestine. *Comp. Biochem. Physiol.* **74A**, 459-462.

**Savory, C. J. and Gentle, M. J.** (1976a). Effects of dietary dilution with fibre on the food intake and gut dimensions of Japanese quail. *Br. Poultry Sci.* **17**, 561-570.

**Savory, C. J. and Gentle, M. J.** (1976b). Changes in food intake and gut size of Japanese quail in response to manipulation of dietary fibre content. *Br. Poultry Sci.* **17**, 571-580.

**Schew, W. A.** (1995). The evolutionary significance of developmental plasticity in growing birds. PhD dissertation, University of Pennsylvania, Philadelphia.

**Schew, W. A. and Ricklefs, R. E.** (1998). Developmental plasticity. In *Avian Growth and Development. Evolution within the Altricial-Precocial Spectrum* (ed. J. M. Starck and R. E. Ricklefs), pp. 288-304. New York, Oxford University Press.

**Spitzer, G.** (1972). Jahreszeitliche Aspekte der Biologie der Bartmeise (*Panurus biarmicus*). *J. Orn.* **113**, 241-275.

**Starck, J. M.** (1996a). Phenotypic plasticity, cellular dynamics, and epithelial turnover of the intestine of Japanese quail (*Coturnix coturnix japonica*). *J. Zool. Lond.* **238**, 53-79.

**Starck, J. M.** (1996b). Intestinal growth in the altricial European starling (*Sturnus vulgaris*) and the precocial Japanese quail (*Coturnix coturnix japonica*). A morphometric and cytokinetic study. *Acta Anat.* **156**, 289-306.

**Starck, J. M.** (1999a). Structural flexibility of the gastro-intestinal tract of vertebrates. Implications for evolutionary morphology. *Zool. Anz.* **238**, 87-101.

**Starck, J. M.** (1999b). Phenotypic flexibility of the avian gizzard: rapid, reversible and repeated changes of organ size in response to changes in dietary fibre content. *J. Exp. Biol.* **202**, 3171-3179.

**Starck, J. M., Abe, A. S. and Neto, A. C.** (2002). Morphological and physiological responses to feeding in broad-nosed caiman (*Caiman latirostris*). *Adv. Ethol. (Suppl. Ethology)* **37**, 118.

**Starck, J. M. and Beese, K.** (2001). Structural flexibility of the intestine of Burmese python in response to feeding. *J. Exp. Biol.* **204**, 325-335.

**Starck, J. M. and Beese, K.** (2002). Structural flexibility of the small intestine and liver of garter snakes in response to feeding and fasting. *J. Exp. Biol.* **205**, 1377-1388.

**Travis, J.** (1994). Evaluating the adaptive role of morphological plasticity. In *Ecological Morphology. Integrative Organismal Biology* (ed. P. C. Wainwright and S. M. Reilly), pp. 99-122. Chicago: University of Chicago Press.

**Uni, Z., Geyra, A., Ben-Hur, H. and Skalan, D.** (2000). Small intestinal development in young chick: crypt formation and enterocyte proliferation and migration. *Br. Poultr. Sci.* **41**, 544-551.

**Williamson, R. C. N. and Chir, M.** (1978). Intestinal adaptation. Structural, functional and cytokinetic changes. *New Engl. J. Med.* **298**, 1393-1402.

**Withers, P. C.** (1977). Measurement of $V_{O_2}$, $V_{CO_2}$, and evaporative water loss with a flow through mask. *J. Appl. Physiol.* **42**, 120-123.