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No effect of short-term exposure to high-fibre diets on the gastrointestinal morphology of layer hens (*Gallus gallus domesticus*): body reserves are used to manage energy deficits in favour of phenotypic plasticity

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Abstract

Using layer hens, *Gallus gallus domesticus*, we compared the digestive capabilities of birds on a low-fibre diet (LF, 8.49% neutral detergent fibre; NDF), with those fed a high-fibre diet balanced for energy and protein to match the LF diet (high fibre balanced, HFB; NDF=15.61%) and those fed a high fibre unbalanced (HFU) diet (NDF=16.68%). The HFU diet had the lowest apparent dry matter (DM) metabolisability at $58.14 \pm 6.46\%$, followed by HFB, 65.87 ± 3.50 and the LF diet, $70.49 \pm 7.07\%$. Despite significant differences between apparent DM metabolisabilities of LF and HFU diets, no morphometric changes in the gastrointestinal tract (GIT) of layer hens were observed (including crop, gizzard, proventriculus, liver, large intestine, paired caeca and small intestine). Conversely, body mass losses were recorded for animals on HFU diet, while those on the LF and HFB diets actually gained body mass over the 14-day trials. We suggest that the body mass losses seen in the animals fed HFU diets were attributed to losses in adipose tissue, but this was not quantified. Assuming body mass losses were mainly in adipose tissue, we propose that adipose may act to buffer environmental challenges like shortfalls in nutrient acquisition when dietary energy requirements are not met. Compared with smaller birds (e.g. quail), the larger body size of the layer hens may offer them a greater safety margin in terms of body energy reserves before changes in the GIT might be needed to redress energy deficits associated with hard-to-digest, high-fibre diets.

Keywords

Phenotypic plasticity, gastrointestinal tract, dietary fibre content, nutrition dilution, neutral detergent fibre, layer hens, *Gallus gallus domesticus*, apparent metabolisability, adipose tissue

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No effect of short-term exposure to high-fibre diets on the gastrointestinal morphology of layer hens (*Gallus gallus domesticus*): body-reserves are used to manage energy deficits in favour of phenotypic plasticity.

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Summary

Using layer hens, *Gallus gallus domesticus*, we compared the digestive capabilities of birds on a low fibre diet (LF, 8.49% neutral-detergent fibre; NDF), with those fed a high-fibre diet balanced for energy and protein to match the LF diet (high fibre balanced, HFB; NDF = 15.61%), and those fed a high fibre, unbalanced (HFU) diet (NDF = 16.68%). The HFU diet had the lowest apparent dry matter (DM) metabolisability at $58.14 \pm 6.46\%$, followed by HFB, 65.87 ± 3.50 , and the LF diet, $70.49 \pm 7.07\%$. Despite significant differences between apparent DM metabolisabilities of LF and HFU diets, no morphometric changes in the gastrointestinal tract (GIT) of layer hens were observed (including: crop, gizzard, proventriculus, liver, large intestine, paired caeca and small intestine). Conversely, body mass losses were recorded for animals on HFU diet, while those on the LF and HFB diets actually gained body mass over the 14-day trials. We suggest that the body mass losses seen in the animals fed HFU diets were attributed to losses in adipose tissue, but this was not quantified. Assuming body mass losses were mainly adipose tissue, we propose that adipose may act to buffer environmental challenges like shortfalls in nutrient acquisition when dietary energy requirements are not met. Compared with smaller birds (e.g. quail), the larger body size of the layer hens, may offer them a greater safety margin in terms of body energy reserves before changes in the GIT might be needed to redress energy deficits associated with hard-to-digest, high fibre diets.

35 **Keywords**

36 Phenotypic plasticity, gastrointestinal tract, dietary fibre content, nutrition dilution,
37 neutral detergent fibre, layer hens, *Gallus gallus domesticus*, apparent metabolisability,
38 adipose tissue.

39

40 **List of Abbreviations**

41 NDF Neutral Detergent Fibre – ADF Acid Detergent Fibre – CF Crude Fibre – DM

42 Dry Matter – OM Organic Matter – LF Low Fibre – HFB High Fibre Balanced – HFU

43 High Fibre Unbalanced – GIT Gastrointestinal Tract – AM Apparent Metabolisability

Introduction

Organisms face various challenges in environmental conditions and internal physiological demands. Some of these challenges may include seasonal fluctuations in food availability and nutrient composition (quality), increased energetic demands during cold seasons, reproduction, or migration, and reduced energetic demands as a result of hibernation or torpor (Starck, 1999a,b; McWilliams and Karasov, 2001; Piersma and Drent, 2003; Starck and Rahmaan, 2003; Munn *et al.*, 2010). One way that animals can deal with such challenges is to alter the size or function of organs, particularly those of the energy-harvesting gastrointestinal system (Starck, 1999a, b; Piersma and Drent, 2003). With respect to the functional capacity of gastrointestinal organs, there are numerous examples of flexible responses to changing conditions, and these can be fast, reversible and repeatable (Piersma and Lindström, 1997; Starck, 1999a, b; McWilliams and Karasov, 2001; Piersma and Drent, 2003; Starck and Rahmaan, 2003). It has been suggested that the functional capacity of an organ that is in excess of its actual demands may be expensive and inefficient, whereas an insufficient functional capacity could limit the ability of the system to accommodate changes in resource quality or availability (Diamond and Hammond, 1992). This is based upon two assumptions: firstly, that an organism has a limited energy budget and under-utilised functional capacities ‘cost’ a measurable portion of the energy budget; and secondly, organisms should respond to environmental and internal demands by matching the functional capacities to avoid reduction in fitness (Diamond and Hammond, 1992; Starck, 1999a, b). In this regard, changes to the functional capacity of digestive organs are of interest because they are the major interface between an organism and its ability to acquire nutrients from the environment.

Fluctuating internal demands or changes in resource availability can change the demands placed on an animal's gastrointestinal tract (GIT). Changes in the size (e.g. mass, length) of GIT organs is one way animals can respond to these fluctuating internal and external conditions, a prime example of phenotypic plasticity (Piersma and Lindström, 1997; Starck, 1999a, b; Starck and Rahmaan, 2003). Such plasticity has been described in various mammals, reptiles, amphibians and birds (Piersma and Lindström, 1997; Starck, 1999a, b; Starck and Rahmaan, 2003). In respect to avian species, transformations have been observed in gross morphometry, such as gizzard size and mass, intestinal length and circumference, and at a cellular level, changes in the dynamics of cellular turnover, cell size and cell proliferation (Starck, 1996; Piersma and Lindström, 1997; Starck, 1999a, b; Starck and Rahmaan, 2003). Here, we investigated the possibility of phenotypic flexibility of a heavily domesticated species, the laying hen, *Gallus gallus domesticus*. Previous studies (e.g. Savory and Gentle 1976; Starck 1999b) have reported flexibility in the gastrointestinal tract of domestic birds (chickens and quail) associated with diets with marked differences in dietary fibre content, chemical composition and dilution of diet energy and protein contents associated with increased fibre contents. However, it is difficult to assess which dietary factors may induce morphometric changes in the avian digestive system from these studies: high fibre or low nutrient density? As such, our study used diets based upon similar ingredients and of similar chemical compositions, with two high fibre diets of fixed dietary fibre contents (wheat bran) and compared with a standard low fibre (LF) layer diet. To account for the effects of dilution via the increased fibre content, one diet of the high fibre diets was balanced, using corn oil, with energy and protein to match the LF diet. To further examine the possible functional trade-offs associated with gut flexibility (e.g. bigger gut = bigger demand, but better digestibility) we quantified hen feed intakes, digestive performance

(dry matter and energy metabolisability), body mass changes and reproductive outputs (egg mass and number).

Materials and methods

Housing and Animal Management

Domestic chicken (*Gallus gallus domesticus*) (n= 18) were obtained from a commercial poultry farm, Albion Park Poultry, Albion Park, New South Wales, Australia. Upon collection, birds were on average 18 months of age and their average body masses were 2.04 ± 0.2 kg. Throughout the acclimation and experimental periods, birds were weighed once weekly to monitor body condition. Only females were used in this study to investigate the effects of digestive plasticity on egg production and to exclude effects of sex. Birds were held in the Ecological Research Centre (ERC) at the University of Wollongong (34°25'S, 150°54'E). Upon introduction to the ERC, birds were treated for internal and external parasites using Piperazine Solution (Piperazine anhydrous: 172 5g L⁻¹; Inca (Flight) Co. Pty Ltd, St Mary's, NSW, Australia) and Pestene-Insect Powder (Sulfur: 50g kg⁻¹, Rotenone: 10g kg⁻¹; Inca (Flight) Co. Pty Ltd, St Mary's, NSW, Australia), birds were treated again upon introduction to individual housing and then treated fortnightly henceforth. During the acclimation period, birds were housed in groups for two weeks to allow birds to habituate to captive conditions; a further ten days were used to acclimatise birds to individual housing and adjust to the control and experimental diets (see Diet Composition and Feeding Trials). Throughout the experimental period, birds were individually housed in standardised stainless steel mesh cages (0.6 m wide x 0.6 m deep x 0.5 m high) equipped with collection trays lined with waxed paper for excreta collection. Each cage was equipped with plastic self feeders for food and water; throughout the entire study food and water were offered *ad libitum*. Birds were housed at temperatures between temperature of 22 – 25°C with air humidity

50 – 60% and a 14 h: 10 h light : dark regime, using full spectrum UV fluorescent light globes, with lights on at 0600 h.

Diet Composition and Feeding Trials

Domestic chickens were assigned randomly to either the control or an experimental diet. Control and experimental diets were supplied in mash form. Prior to the experimental period, birds were fed a standard commercial layer pellet ('Back Yard Layer' - The Vella Group, Plumpton, NSW, Australia) containing 8.37% as fibre as fed (fibre type not defined). Upon introduction and acclimation to individual housing, birds were fed the control diet. During the acclimation period to experimental diets, birds were acclimated to a high fibre experimental diet (balanced or unbalanced) with a transition period where the experimental diet was systematically introduced (0%, 30%, 50%, 70%, 100%) to the control diet across a five day period.

The control and two experimental diets were obtained from the Poultry Research Foundation of the University of Sydney, Camden, New South Wales, Australia. The birds were fed one of three diets, low fibre (LF; control diet), high fibre balanced (HFB) or high fibre unbalanced (HFU) (see Table 1 for composition). Fibre for the experimental diets was sourced from wheat bran. All diets were analysed to determine proximate chemical composition (See Sample Analysis).

Body Mass, Feed Intake, Egg Production and Apparent Metabolisability

Body mass, food intake and eggs laid were measured daily; all masses were measured in grams, with the exception of body mass being measured in kilograms. Feed remaining and spilt food were collected quantitatively and stored in a dry air tight room (see

Sample Analysis). After the 14 day experimental period, birds were euthanased to measure morphometric differences.

The intake of dietary components (Dry Matter Intake) was calculated as [(Feed offered – Feed Remaining and Spilt)*%Dry Matter]. The apparent and energy metabolisabilities (%) of the dietary components were calculated as:

$$\left[\frac{((\text{Dry Matter Intake} - \text{Dry Matter Output}) / \text{Dry Matter Intake})}{100} \right] * 100$$
 and
$$\left[\frac{((\text{Dry Matter Energy Intake} - \text{Dry Matter Energy Output}) / \text{Dry Matter Energy Intake})}{100} \right] * 100$$
 respectively, where Intake and Output were measured as g per chicken day⁻¹ (Robbins, 1993).

Dissections and Morphometry

Animals were euthanased using isoflurane overdose followed by cervical dislocation. Immediately following euthanasia, macroscopic dissections and measurements of organ size were conducted to study morphometric differences between control and experimental groups. Organs (liver, crop, proventriculus, gizzard, small intestine, left and right caeca and large intestine) were weighed using scales with ± 0.01 g precision, and measured using slide callipers with ± 0.05 mm precision. Wet mass, with and without digesta contents were recorded, organs were emptied and rinsed with a 0.9 % saline solution and re-weighed. Organs then were air dried in an oven at 70°C until a constant weight to determine dry masses.

Sample Analysis

Daily samples of food offered, food remaining, food spilt and faecal output were collected and weighed to determine wet mass. Faecal samples were stored frozen (-20°C). Faecal samples from each day were then later thawed and sub samples (approximately 25% wet mass) along with sub-samples of food offered were prepared (approximately 25% wet mass) for analysis by air drying in an oven at 103°C for 48h (Robertson and Van Soest, 1981) to determine dry masses.

The dried samples of feed offered were ground using a Wiley Mill through a 1 mm mesh (Arthur Thomas Co. Scientific Apparatus, Philadelphia, USA). Ash content of the dried, ground feed offered were determined by dry ashing 0.5 g samples in a Thermolyne Muffle Furnace (Model 62700; Dubuque, Iowa, USA) at 550°C for 12 h. Organic matter of food stuffs was calculated as the Dry Matter content – Ash Content.

The Neutral Detergent Fibre (NDF; comprised of mainly cellulose, hemicellulose and lignin) and Acid Detergent Fibre (ADF; comprised of mostly cellulose and lignin) of the three diets offered were determined using reagents and procedures as described by Van Soest *et al.* (1991); before neutral detergent digestion, duplicate 0.5 g sample bags were washed in acetone to remove any soy bean products from the extract samples. Using a sequential filter bag technique, to reduce any unintentional loss of samples during the procedure, and ANKOM Fibre Analyser (Model 220, ANKOM Technology Corp., Fairport New York, USA), the soluble cell contents (calculated as Dry Matter – Neutral Detergent Fibre), hemicellulose (NDF – ADF) and cellulose contents (ADF – lignin) in the three diets were determined by the difference. NDF and ADF were not adjusted for ash content and are presented on a %DM basis only.

The energy content of the dried, ground feed and faeces were determined via the combustion of duplicate ca. 0.5 g sub samples in a bomb calorimeter (Gallenkamp, Model CB-375; Gallenkamp and Co. Ltd, Loughborough, UK); A standard benzoic acid was used for calibration. Nitrogen content of feed was determined by total combustion of duplicate 0.2 g samples in a Leco CHN-1000 elemental analyser (Leco Inc. St Joseph, Michigan, USA).

Statistical Analyses

Values for gut morphometric data, food intake, apparent metabolisability, body condition and egg production were expressed as mean $\pm$ S.D. unless stated otherwise. Univariate analysis of covariance (ANCOVA; Quinn and Keough, 2002) with body mass as covariate were used to evaluate the effects of diet on feed intake, apparent and energy metabolisabilities, egg production and gut morphometric parameters, and for the digestive tract as a whole and evaluation for individual digestive organs, taken as wet and dry masses. Egg production was measured as egg mass, number of eggs produced, frequency of eggs laid and reproductive output index (measured as Egg mass * Number of eggs produced) throughout the experimental period.

To test for differences between feed intake in the acclimation and experimental periods, measured as a significant increase or decrease of intake for each diet, firstly a two tailed Z test was used, with an expected mean of 84.3 g day⁻¹ (the mean food intake of all individuals in the acclimation period). Once determined significantly different, an ANCOVA with body mass as covariate was run to evaluate the effects of diet on food intake difference. A further Z-test and ANCOVA, following the above methodology with an expected mean feed intake change of 0.0 g, was used to determine whether there

were any significant differences between feed intake in the initial and final stages of the experimental period.

To investigate the effects of diet on body condition, an analysis of variance (ANCOVA) with body mass as a covariate was used. To evaluate the difference of body mass from the initial body mass, measured as a significant loss or gain of body masses for animals on each diet, firstly was examined using a two tailed Z-test, with an expected mean of 0.00 kg (i.e., no change in body mass). Once determined significantly different, an ANOVA was run to evaluate the effects of diet on weight difference.

To meet assumptions of ANCOVA, normality was tested using Shapiro Wilks test ($\alpha=0.05$) and Levene's for homogeneity of variance ($\alpha=0.05$). To meet normality and/or homogenous variance, $\log_{10}$ transformations were used on the following data sets: Small Intestine empty wet mass (g) and dry matter intake during acclimation period (g day^{-1}); Arc Sine transformation was used on apparent metabolisability (Zar, 1999) and a log transformation used on the Proventriculus dry mass. To achieve normality for data investigating egg production, one individual was omitted because it did not lay throughout the entire experiment. Left and right caeca empty wet and dry masses (g) were combined to account for an observed deviation in caecal morphogenesis observed in one bird (S.K. Courtney Jones, unpublished), combined caecal empty wet mass was noted to achieve normality and homogeneity of variance. However, normality for combined caecal dry mass was not met, it was assumed the robustness of the ANCOVA would suffice. Significant differences that were detected by the ANCOVA were further examined using a Tukey's Honest Significant Difference (HSD) *post hoc* test. Data collected were analysed in JMP 7.0.2 statistical package (SAS Institute Inc, 2007).

Results

Body Condition and Food Intake

The body mass of *G. gallus domesticus* on the control and experimental diets taken as an average during the experimental period (14 days) were determined not significantly different, with a mean body mass of 2.07 ± 0.28 , 2.02 ± 0.31 and 2.00 ± 0.27 kg throughout the experimental period (LF, HFB and HFU diets respectively; $F= 0.072$; $p= 0.93$; $n= 18$). It was noted, however not quantified in this study, that high amounts of adipose tissue surrounded the lower abdomen (S.K. Courtney Jones, pers. obs.).

During the experimental period, the body mass of birds on low fibre (LF) and high fibre balanced (HFB) diets had significantly increased from the initial body mass (based upon average acclimation period body mass) at the conclusion of the experimental period by 0.07 ± 0.08 kg and 0.05 ± 0.06 kg respectively (LF: $z= 2.775$, $p= 0.031$; HFB: $z= 2.704$, $p= 0.026$; Table 2); however this gain of body mass was not significantly different between LF and HFB animals (ANCOVA with body mass as covariate; d.f.= 2, 17; $F= 5.96$; $p= 0.005$; body mass was not significant as covariate; see Table 2). In contrast, the mean body mass of birds on the high fibre unbalanced (HFU) had significantly declined from the initial body mass by the concluding day of the experimental period, mean body mass loss was 0.08 ± 0.08 kg, significantly different from a change of zero ($z= -2.479$, $p= 0.015$; see Table 2) and was significantly different from the LF and HFB diets (ANCOVA with body mass as covariate; d.f.= 2, 17; $F= 5.96$; $p= 0.005$; body mass was not significant as covariate; see Table 2).

During the acclimation period, birds ingested an average of 84.30 ± 14.21 grams ($n = 18$) of the control (LF) diet per day; the transition into experimental period (100% as fed control or experimental diets) did not significantly increase or decrease dry matter (DM)

intake compared to the quantities consumed during the acclimation period (LF: $z = 0.336$, $p = 0.189$; HFB: $z = 0.295$, $p = 0.76$; HFU: $z = 0.593$, $p > 0.05$; see Table 1). Furthermore, the diet composition of control and experimental diets did not significantly affect DM feed intake per day, during the experimental period (ANCOVA with body mass as covariate; d.f. = 2, 17; $F = 3.90$; $p = 0.713$; body mass was not significant as covariate; see Table 2). From the initial feed intake during the experimental period, birds on the LF and HFB diets decreased their final DM feed intake by 13.44 ± 27.08 and 11.43 ± 15.83 grams respectively; in contrast, birds on the HFU diet increased their DM feed intake by 3.57 ± 24.24 grams, however due to high inter-individual variability during the experimental period, the changes in DM feed intake were not statistically significant (LF: $z = -4.936$, $p = 0.003$; HFB: $z = -3.395$, $p < 0.001$; HFU: $z = 16.647$, $p < 0.001$; ANCOVA with body mass as covariate; d.f. = 2, 17; $F = 0.693$; $p = 0.639$; body mass was not significant as covariate).

Apparent Metabolisability and Energy Metabolisability

Apparent metabolisability (%) of the dietary dry matter (DM) was calculated at 70.49 ± 7.07 , 65.87 ± 3.50 and 57.14 ± 6.46 % for LF, HFB and HFU respectively; a statistical difference occurred only between the LF and HFU apparent metabolisabilities with HFU approximately 17.5% lower than the LF diet (ANCOVA with body mass as covariate; d.f. = 2, 17; $F = 5.472$; $p = 0.021$; body mass was not significant as covariate; see Table 2). Energy metabolisability (%) of the HFU diet was significantly lower than that of the LF or HFB diet, with HFU approximately 13% lower than the LF diet (ANCOVA with body mass as covariate; d.f. = 2, 17; $F = 5.907$; $p = 0.0164$; body mass was not significant as covariate; see Table 2).

Gut morphometry

Overall, diet composition and fibre content in the high fibre diets (HFB and HFU) had no significant effect on the empty wet masses or dry masses of the total digestive tract of hens (empty wet mass: ANCOVA with body mass as covariate; d.f.= 2, 17; F= 0.267; p= 0.922; body mass was not significant as a covariate; dry mass: ANCOVA with body mass as covariate; d.f.= 2, 17; F= 3.499; p= 0.064; body mass was not significant as a covariate). Individually weighed digestive organs including crop, proventriculus, gizzard, small intestine, combined left and right caeca and large intestine did not significantly differ in mean empty wet masses or dry masses between control and experimental groups (ANCOVA with body mass as covariate; d.f. = 2, 17; body mass was not significant as a covariate; see Table 3 and 4 for F and P values). Furthermore, left caeca, right caeca and small intestine lengths did not significantly differ between experimental groups (ANCOVA with body mass as covariate; d.f.= 2, 17; body mass was not significant as a covariate; see Table 3 for F and P values).

Egg Production

Diet composition and fibre content did not have a significant effect on the egg mass, as eggs produced by *G. gallus domesticus* on the control and experimental diets had a mean egg mass of 64.63 ± 1.73 , 64.45 ± 4.71 and 64.38 ± 4.74 g throughout the experimental period (LF, HFB and HFU diets respectively; ANCOVA with body mass as covariate; d.f.= 2, 16; F= 4.098; p= 0.832; body mass was not significant as covariate; n= 17; see Table 5). Number of eggs produced was not significantly different between diet groups with a mean 10.17 ± 1.17 , 9.8 ± 1.92 and 8.50 ± 2.88 eggs produced throughout the entire experimental period (LF, HFB and HFU diets respectively; 14 days; ANCOVA with body mass as covariate; d.f.= 2, 16; F= 0.882; p= 0.542; body mass was not significant as covariate; n= 17). Frequency of eggs (number of eggs produced per day

within experimental period) also was not significantly affected by dietary composition, with mean frequency of 0.73 ± 0.08 , 0.70 ± 0.14 and 0.61 ± 0.21 eggs laid per day (LF, HFB and HFU diets respectively; ANCOVA with body mass as covariate; d.f. = 2, 16; $F = 0.882$; $p = 0.524$; body mass was not significant as covariate; $n = 17$). Further, the reproductive output index between experimental groups did not significantly differ (ANCOVA with body mass as covariate; d.f.= 2, 16; $F = 1.084$; $p = 0.421$; body mass was not significant as covariate; $n = 17$).

Discussion

Phenotypic flexibility of the gastrointestinal tract has been reported for a variety of bird species in response to variations in diet fibre content (Starck, 1999a, b). However, we found no effect of high fibre contents on the gut morphometry of layer hens (Table 3 and 4). We also observed no statistically significant differences in mean DM or energy intake or egg production of hens in response to high fibre diets (HFU or HFB) compared with those offered LF diets. This was unexpected, but raises interesting questions regarding the role of body size, and specifically body reserves in birds, and whether these reserves may be preferentially utilised in favour of major gut re-modelling to manage poor-quality diets.

Starck and Rahmaan (2003) investigated gastrointestinal flexibility and resting metabolic rates (RMR) in Japanese Quail (*Corturnix japonica*) in response to high fibre diets, it was noted that quail lost adipose tissue in response to a 40% non-digestible-fibre diets. Starck and Rahmaan (2003) suggested that quails fuelled the plasticity of the gastrointestinal tract by mobilisation of the lipid stores in liver and adipose tissue, but maintained their resting/basal metabolic rates. However, in contrast to Starck and Rahmaan (2003), birds in this present study did not exhibit changes in the

gastrointestinal tract, suggesting that adipose tissue and bone reserves could play a preliminary buffering role in responding to environmental and/or dietary challenges, and particularly for managing reduced energy intakes associated with increased contents of hard-to-digest fibre.

The hens in our experiment offered the HFU diet did indeed have lower overall energy intakes and metabolisabilities compared with those fed LF or HFB diets (Tables 1 and 2), but phenotypic changes in the gut were not used to redress this energy deficit and, presumably, some other compromise in the hens energy budget had occurred. This suggestion is supported when examining our apparent metabolisability data along with the body mass losses observed for the hens offered HFU diets. Large deposits of intra-abdominal fat surrounded the gastrointestinal tract of all hens across all diet treatments, and this may have supported hens fed HFU diets during the feeding trial (Table 2). For example, if we assume that the body mass losses observed for our hens offered HFU diets was entirely comprised of body fat (average losses = 80 g over 14 days; Table 2) and that fat contains 38 kJ g⁻¹ (Wither, 1992), a total 3,040 kJ could have been recovered (assuming a 100% conversion efficiency). Given that the total energy deficit of the hens offered HFU diet over 14 days was 3,243 kJ relative to hens offered the LF diet, it is apparent that their body reserves could have supported egg productivity throughout; egg production was no different between the hens offered low or high fibre diets (Table 5)

Other studies have indicated that domestic hens can preserve egg production in favour of body mass when faced with energy deficits or other environmental stressors (Taylor and Moore, 1954; Harms *et al.* 2000; Leeson *et al.* 2001; Usayran *et al.* 2001). Harms *et al.* (2000) and Leeson *et al.* (2001), for example, demonstrated that egg production was independent of dietary energy level, however when energy intake was deficient, a

decline in egg production was eventually observed. Usayran *et al.* (2001) observed that layer hens under high environmental temperatures directed nutrients to support egg production rather than maintaining body mass. Notably, Deaton *et al.* (1977) found that layer hens fed high fibre diets increased their gizzard weight and also maintained egg production compared with those fed lower fibre diets, however such changes were only exhibited across a 12-month experimental period with 8.07% crude fibre content (Deaton *et al.*, 1977). This generates the interesting idea that digestive plasticity may dependant on the amount of body reserve, rather than a direct response to the structure or composition of diets themselves. An important question then becomes, at what level of body-reserve depletion might the system switch from using reserve to buffer energy deficits directly, and when might it be used to fuel flexible changes in the gastrointestinal tract? Studies on the interplay between phenotypic plasticity and body reserves are lacking.

That we found no morphometric changes in the gut of layer hens in response to high fibre contents is somewhat unusual. Changes in the gut of numerous smaller birds, including young chickens, offered high-fibre diets have been reported repeatedly (e.g. Kondra *et al.*, 1974; Caviedes-Vidal and Karasov, 1996; Starck, 1999b; Starck and Rahmaan, 2003; Brzek *et al.*, 2011). These studies have been able to induce gut changes in these smaller birds (e.g. Japanese quail, *Coturnix japonica*; house sparrow, *Passer domesticus*; and Zebra Finch, *Taeniopygia guttata*) sometimes within days. The lack of changes in our layer hens, which are larger birds, raises allometric scaling as a factor worth considering in the role of body-reserves mediating gut plasticity. Larger animals appear capable of sustaining biological processes during prolonged nutritional deficits compared with smaller animals (Barboza and Hume 2006). This is attributed to the smaller animals having relatively higher maintenance requirements for energy and

protein in comparison to larger animals (Barboza and Hume, 2006), and that larger animals have relatively greater stores of body protein and lipids (Calder 1984). Smaller animals, therefore, usually have lower stores of body protein and lipids on which to draw to manage energy/nutrient deficits, and thus smaller animals are likely more susceptible to hypophagia in comparison to larger animals. Therefore, while changes in the size of digestive organs in response to high fibre diets have been reported for numerous small birds (Caviedes-Vidal and Karasov 1996; Starck 1999b; Starck and Rahmaan 2003; Brzek *et al.*, 2011;), the lack of similar findings for our larger, mature layer hens suggest that a larger body size, and extensive body fat, supports short-term nutrient deficits in favour of gut changes. Presumably, if our hens were fed the HFU diet for a longer period, gut changes may have occurred, but this hypothesis remains to be tested.

Understanding what drives changes in the GIT in response to diet quality or other environmental challenge is important, but the mechanisms controlling plasticity are uncertain (Starck 1999a). Our data suggest that the level of body reserves may be involved, but we also highlight a limitation of previous studies. Notably, previous studies that have investigated the effects high fibre contents have typically diluted standard, high-quality (low fibre) diets with high fibre sources (e.g. wheat bran, saw dust; Jørgensen *et al.*, 1996; Starck, 1999b and Starck and Rahmaan, 2003), thereby diluting available energy and other nutrients. Our study controlled for diet dilution, however, by using a high fibre diet balanced for energy and protein to match a low fibre diet (i.e. HFB diet). A key finding from this approach was that the energy metabolisability of the LF and HFB diets by our layer hens were not significantly different from each other, but were each significantly higher than that of hens fed the HFU diet. This finding suggests that the gastrointestinal changes observed in smaller bird species fed high-fibre diets (e.g. Jørgensen *et al.*, 1996; Starck, 1999b and Starck

and Rahmaan, 2003) may have been associated primarily with nutrient dilution, rather than any direct effect of the higher fibre content per se.

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References

- Barboza, P.S and Hume, I.D. (2006) Physiology of Intermittent Feeding: Integrating Responses of Vertebrates to Nutritional Deficit and Excess. *Physiological and Biochemical Zoology* **79**: 250 – 264.
- Brzek, P., Kohl, K.D., Caviedes-Vidal, E. and Karasov, W.H. (2011) Fully reversible phenotypic plasticity of digestive physiology in young house sparrows: lack of long-term effect of early diet composition. *The Journal of Experimental Biology* **214**: 2755 – 2760.
- Calder W.A.I. (1984) *Size, Function, and Life History*. Harvard University Press, Cambridge, UK.

451 Caviedes-Vidal, E. and Karasov, W.H. (1996) Glucose and amino acid absorption in
 452 house sparrow intestine and its dietary modulation. *The American Journal of*
 453 *Physiology* **271**: 561 – 568.

454 Diamond, J. M. and Hammond, K. A. (1992) The matches, achieved by natural
 455 selection, between biological capacities and their natural loads. *Experientia* **48**:
 456 551-557.

457 Deaton, J.W., Kubena, L.F., Reece, F.N. and Lott, B.D. (1977) Effect of dietary fibre on
 458 the performance of laying hens. *British Journal of Poultry Science* **18**: 711 – 714.

459 Harms, R. H., Russell, G.B. and Sloan, D.R. (2000) Performance of four strains of
 460 commercial layers with major changes in dietary energy. *Journal of Applied*
 461 *Poultry Research* **9**: 535 – 541.

462 Jørgensen, H., Zhao, X-Q, Knudsen, K.E.B. and Eggum, B.O. (1996) The influence of
 463 dietary fibre source and level on the development of the gastrointestinal tract,
 464 digestibility and energy metabolism in broiler chickens. *British Journal of*
 465 *Nutrition* **75**: 379 – 395.

466 Kondra, P.A., Sell, J. L. and Guenther, W. (1974) Response of meat- and egg-type
 467 chickens to a high fibre diet. *Canadian Journal of Animal Science* **54**: 651 -658.

468 Leeson, S., Summers, J.D. and Caston, L.J. (2001) Response of layers to low nutrient
 469 density diets. *Journal of Applied Poultry Research* **10**: 46 – 52.

470 McWilliams, S. R. and Karasov, W. H. (2001). Phenotypic flexibility in the digestive
 471 system structure and function in migratory birds and its ecological significance.
 472 *Comparative Biochemistry and Physiology - Part B* **128**: 579 – 593.

473 Miller, M.R. (1975) Gut morphology of Mallards in relation to diet quality. *Journal of*
 474 *Wildlife Management* **39**: 168 – 173.

475 Munn, A.J., Banks, P. and Hume, I.B. (2006) Digestive plasticity of the small intestine
 476 and the fermentative hindgut in a marsupial herbivore, the tammar wallaby
 477 (*Macropus eugenii*). *Australian Journal of Zoology* **54**: 287 – 291.

478 Munn, A.J., Kern P., McAllan, B.M. (2010) Coping with chaos: unpredictable food
 479 supplies intensify torpor use in an arid-zone marsupial, the fat-tailed dunnart
 480 (*Sminthopsis crassicaudata*). *Naturwissenschaften* **97**: 601 – 605.

481 Piersma, T. and Lindström, A. (1997) Rapid, reversible changes in organ size as a
 482 component of adaptive behaviour. *Trends in Ecology and Evolution* **12**: 134 –
 483 138.

484 Piersma, T. and Drent, J. (2003) Phenotypic flexibility and the evolution of organismal
 485 design. *Trends in Ecology and Evolution* **18**: 228 – 233.

486 Quinn G. P. & Keogh M. J. (2002) *Experimental Design and Analysis for Biologists*.
 487 Cambridge University Press, Cambridge, UK.

488 Robbins, C. T. (1993) *Wildlife Feeding and Nutrition* (2nd ed.). Academic Press, San
 489 Diego, USA.

490 Robertson, J. B. and Van Soest, P. J. (1981). The detergent system of analysis and its
 491 application to human foods. In *The Analysis of Dietary Fibre in Food* (ed. W. P.
 492 T. James and O. Theander), pp. 123-158. Marcel Dekker, New York, USA.

493 Savory, C. J. and Gentle, M. J. (1976). Effects of dietary dilution with fibre on the food
 494 intake and gut dimensions of Japanese quail. *British Poultry Science* **17**: 561 –
 495 570.

496 Schmidt-Neilson, K. (1984) *Scaling, why is animal size so important?* Cambridge
 497 University Press, Cambridge, UK.

498 Starck, J. M. (1996) Phenotypic plasticity, cellular dynamics, and epithelial turnover of
 499 the intestine of Japanese quail (*Coturnix coturnix japonica*). *Journal of Zoology*:
 500 *Proceedings of the Zoological Society of London* **238**: 53 – 79.

501 Starck, J. M. (1999a) Structural Flexibility of the Gastro-Intestinal Tract of Vertebrates-
502 Implications for Evolutionary Morphology. *Zoologischer Anzeiger*, 238(1-2), 87-
503 101.

504 Starck, J.M. (1999b) Phenotypic flexibility of the avian gizzard: Rapid, reversible and
505 repeated changes of organ size in response to changes in dietary fibre content. *The*
506 *Journal of Experimental Biology* **202**: 3171 -3179.

507 Starck, J.M. and Rahmaan, G.H.A. (2003) Phenotypic flexibility of structure and
508 function of the digestive system of Japanese quail. *The Journal of Experimental*
509 *Biology* **206**: 1887 -1897.

510 Taylor, T.G. and Moore, J.H. (1954) Skeletal Depletion in Hens Laying on a Low-
511 calcium Diet. *British Journal of Nutrition* **8**: 112 – 124.

512 Usayran, N. Farran, M.T., Awadallah, H.H.O., Al-Hawi, I.R., Asmar, R.J. and
513 Ashkarian, V.M. (2001) Effects of Added Dietary Fat and Phosphorus on the
514 Performance and Egg Quality of Laying Hens Subjected to a Constant High
515 Environmental Temperature. *Poultry Science* **80**: 1695 - 1701.

516 Van Soest, P.J., Robertson, J.B. and Lewis, B.A. (1991) Methods for Dietary Fiber,
517 Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal
518 Nutrition. *Journal of Dairy Science* **74**: 3583 – 3597.

519 Wither, P.C. (1992) *Comparative Animal Physiology*. Saunders College Publishing,
520 Philadelphia, USA.

521 Zar, J. H. (1999) *Biostatistical Analysis* (4th ed.). Prentice-Hall Inc, Sydney, Australia.
522

523 Table 1: Ingredient and proximate nutrient composition of the experimental diets.

	Diet Low Fibre (Balanced)	High Fibre (Balanced)	High Fibre (Unbalanced)
<i>Ingredients (%)</i>			
Wheat – Feed	72.5	44.6	54.4
Soybean Meal 48	15.2	13.2	11.4
Wheat Bran	-	25.0	25.0
Corn Oil	2.1	7.1	1.6
Salt	0.14	0.17	0.11
Sodium Bicarbonate	0.21	0.17	0.16
DL Methionine	0.08	0.08	0.06
Lysine HCl	0.02	0.00	0.02
Limestone	8.8	8.8	6.6
Dicalcium Phosphate	0.77	0.77	0.58
Vitamin Premix	0.20	0.20	0.15
<i>Chemical Analyses</i>			
Dry Matter (%) [*]	92.1	92.0	93.0
Organic Matter (%) [*]	90.9 ± 0.1	86.7 ± 0.03	89.3 ± 0.1
Nitrogen (%) [*]	3.1	3.0	3.0
Crude Protein (%) [*]	19.2	18.5	18.8
Gross Energy (kJg ⁻¹ DM as fed) [*]	16.4 ± 0.2	18.5 ± 0.8	17.2 ± 0.4
Metabolisable Energy (kJd ⁻¹ DM) ^{**, #}	11.5 ± 1.2 ^a	12.2 ± 0.7 ^a	10.0 ± 1.1 ^b
Neutral Detergent Fibre (%) [*]	8.5±0.5	15.6 ± 0.5	16.7 ± 2.5
Acid Detergent Fibre (%) [*]	3.0±0.01	5.9 ± 0.2	6.9 ± 0.1
524	[*] - calculated as dry matter, ^{**} - calculated values, [#] - see Table 2 for F- and P-values		
525	<i>Post-hoc</i> test (ANOVA, Tukey's HSD) for differences among means; means labelled		
526	with differing letters are significantly different.		

Table 2: Dry matter intake and outputs, apparent metabolisabilities and body mass measurements¹

	Diet			F ratio	P-value ²
	Low Fibre	High Fibre (Balanced)	High Fibre (Unbalanced)		
DM Feed Intake per day (g)	100.0 ± 12.5	85.6 ± 10.3	84.1 ± 20.4	0.346	0.713
Acclimation DM Feed Intake per day (g)	82.7 ± 8.5	78.2 ± 10.2	92.0 ± 19.8	1.508	0.258
<i>Initial DM Feed Intake [Day 0] (g)</i>	109.4 ± 13.4	95.2 ± 14.1	95.6 ± 23.9	₋₃	₋₃
<i>Final DM Feed Intake [Day 14] (g)</i>	96.0 ± 17.0	83.7 ± 12.3	99.6 ± 28.1	₋₃	₋₃
DM Feed Intake Difference [Experimental – Acclimation periods] (g)	6.7 ± 12.5	1.3 ± 10.3	-0.2 ± 20.4	₋₄	1.0
DM Feed Intake Difference [Within Experimental period] (g)	-13.4 ± 27.1	-11.4 ± 15.8	3.6 ± 24.2	0.693	0.405
DM Faecal Output per day (g)	26.5 ± 4.5	29.0 ± 5.6	34.4 ± 8.3	2.470	0.092
Metabolisable Energy (kJd ⁻¹ DM) ^{**}	11.5 ± 1.2 ^a	12.2 ± 0.7 ^a	10.0 ± 1.1 ^b	6.262	0.014
Apparent DM Metabolisability (%)	70.5 ± 7.1 ^a	65.8 ± 3.5 ^{ab}	58.1 ± 6.5 ^b	5.472	0.021
Energy Metabolisability Coefficient (%) [*]	72.8 ± 4.9 ^a	72.8 ± 2.8 ^a	63.1 ± 6.9 ^b	5.907	0.016
Body Mass (kg)	2.06 ± 0.28	2.02 ± 0.31	2.00 ± 0.27	0.072	0.931
<i>Initial Body Mass [Day 0] (kg)</i>	2.09 ± 0.25	2.01 ± 0.27	2.06 ± 0.21	₋₅	₋₅
<i>Final Body Mass [Day 14] (kg)</i>	2.10 ± 0.27	2.05 ± 0.29	1.98 ± 0.26	₋₅	₋₅
Body Mass Difference (kg)	0.07 ± 0.08 ^a	0.05 ± 0.06 ^a	-0.08 ± 0.08 ^b	5.963	0.005

¹ – N = 18, d.f. = 2, 17 for all measurements.

² – Probability values of the overall effect of diet with body mass as covariate.

³ – Not statistically analysed, used to determine DM intake difference within experimental period.

⁴ – Z scores given, see Body Condition and Food Intake section in Results.

⁵ – Not statistically analysed, used to determine body mass difference within experimental period.

Post-hoc test (ANOVA, Tukey's HSD) for differences among means; means labeled with differing letters are significantly different.

Table 3: Effect of diet composition on wet gut morphometry (empty wet mass; ANCOVA with body mass as covariate) ¹

	Diet			F ratio	P-value ²
	Low Fibre	High Fibre (Balanced)	High Fibre (Unbalanced)		
Combined Gut (g)	123.7 ± 16.5	131.0 ± 14.2	127.2 ± 11.5	0.267	0.922
Crop (g)	6.4 ± 1.1	7.5 ± 1.6	7.0 ± 1.4	0.482	0.782
Proventriculus (g)	8.2 ± 0.7	9.2 ± 1.5	8.1 ± 0.7	1.004	0.456
Gizzard (g)	26.5 ± 4.6	29.6 ± 4.5	27.9 ± 4.6	0.518	0.757
Small Intestine (g)	31.2 ± 10.2	35.8 ± 4.8	32.1 ± 3.3	0.512	0.762
Large Intestine (g)	3.4 ± 0.6	3.5 ± 0.8	4.12 ± 1.11	0.869	0.529
Combined Caeca (g)	7.0 ± 1.2	7.7 ± 0.8	7.0 ± 0.5	0.688	0.641
Liver (g)	41.0 ± 5.5	38.4 ± 5.2	41.1 ± 8.7	1.052	0.432
Caeca L (g)	3.5 ± 0.5	3.5 ± 1.0	3.4 ± 0.1	0.014	0.999
Caeca R (g)	3.5 ± 0.8	3.5 ± 1.2	3.6 ± 0.5	0.227	0.943
Left Caeca Length (mm)	176.0 ± 36.4	194.7 ± 20.7	185.3 ± 17.2	2.303	0.109
Right Caeca Length (mm)	175.5 ± 29.1	190.3 ± 17.1	189.8 ± 25.5	2.092	0.136
Small Intestine Length (mm)	1387 ± 113	1359 ± 204	1385 ± 923	0.424	0.823

¹ – N = 18, d.f. = 2, 17 for all measurements

² – Probability values of the overall effect of diet with body mass as covariate.

Table 4: Effect of diet composition on dry gut morphometry (empty dry mass; ANCOVA with body mass as covariate)¹

	Diet			F ratio	P-value ²
	Low Fibre	High Fibre (Balanced)	High Fibre (Unbalanced)		
Combined Gut (g)	32.0 ± 4.7	27.9 ± 4.2	28.3 ± 3.1	3.499	0.577
Crop (g)	1.89 ± 0.64	1.60 ± 0.3	1.72 ± 0.4	0.472	0.902
Proventriculus (g) ³	2.69 ± 0.56	2.5 ± 0.4	2.4 ± 0.3	0.538	0.394
Gizzard (g)	9.25 ± 1.65	8.8 ± 1.1	9.2 ± 1.4	0.170	0.927
Small Intestine (g)	13.32 ± 2.29	12.0 ± 2.8	12.1 ± 2.0	1.055	0.407
Large Intestine (g)	0.85 ± 0.24	0.86 ± 0.2	1.05 ± 0.21	1.581	0.194
Combined Caeca (g)	2.00 ± 0.42	2.22 ± 0.45	1.90 ± 0.21	1.120	0.556

¹ – N = 18, d.f. = 2, 17 for all measurements;

² – Probability values of the overall effect of diet with body mass as covariate;

³ – Log transformation on empty dry Proventriculus masses to meet the assumptions of homogeneity of variance

Table 5: Egg production during the 14-day experimental period¹

	Diet			F value	P value ²
	Low Fibre	High Fibre (Balanced)	High Fibre (Unbalanced)		
No. of Eggs during experiment period	10.2 ± 1.2	9.5 ± 1.9	8.6 ± 3.2	0.882	0.524
Egg mass (g)	64.6 ± 1.7	64.3 ± 4.2	64.5 ± 5.3	4.098	0.832
Frequency of eggs laid (eggs per day)	0.70 ± 0.10	0.67 ± 0.13	0.61 ± 0.23	0.882	0.524
Reproductive output (Egg mass (g)*no. of eggs)	656 ± 59	633 ± 64	547 ± 59	1.085	0.421

¹ – N = 17, d.f. = 2, 16 for all measurements

² – Probability values of the overall effect of diet with body mass as covariate.