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Poor welfare or future investment?
Different growth pattern of broiler breeders

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Sammanfattning/Abstract:

The parental stock of meat type chickens (broiler breeders) are commonly feed restricted to decrease their rapid growth and the issues associated with it. Among these birds, chronic hunger and stress are the most prominent welfare concerns and mass heterogeneity within flocks a major management challenge. The present study compared small and large broiler breeders of the same age within a flock, with the hypothesis that small birds would show signs of poorer welfare indicated by higher corticosterone concentration and heterophil/lymphocyte ratio as a consequence of higher experienced feed restriction due to competition. It also aimed to characterize morphometric differences between small and large birds within flocks as well as between birds on different feeding regimens; skip-a-day vs. every-day-fed. Heterophil/lymphocyte ratio at 4 weeks was significantly higher in large birds compared to small birds, but corticosterone concentration did not differ. Relative mass of the upper gastrointestinal tract, pancreas and liver of small birds at 4 weeks of age were significantly larger, while relative muscle and gizzard fat mass were significantly lower compared to large birds. 12 weeks old skip-a-day fed birds largely followed the pattern of 4 weeks old small birds. In the present study, no clear signs of poorer welfare in small broiler breeders could be seen and the morphometric differences might suggest different ways to cope with feed competition. A larger gastrointestinal tract might indicate long-term investments and maybe that smaller broiler breeders, and skip-a-day fed birds, are better habituated to feed restriction.

Nyckelord/Keyword:

Chicken, broiler breeder, welfare, stress, hunger, feed restriction, skip-a-day, morphometrics.

Contents

1 Abstract

The parental stock of meat type chickens (broiler breeders) are commonly feed restricted to decrease their rapid growth and the issues associated with it. Among these birds, chronic hunger and stress are the most prominent welfare concerns and mass heterogeneity within flocks a major management challenge. The present study compared small and large broiler breeders of the same age within a flock, with the hypothesis that small birds would show signs of poorer welfare indicated by higher corticosterone concentration and heterophil/lymphocyte ratio as a consequence of higher experienced feed restriction due to competition. It also aimed to characterize morphometric differences between small and large birds within flocks as well as between birds on different feeding regimens; skip-a-day vs. every-day-fed. Heterophil/lymphocyte ratio at 4 weeks was significantly higher in large birds compared to small birds, but corticosterone concentration did not differ. Relative mass of the upper gastrointestinal tract, pancreas and liver of small birds at 4 weeks of age were significantly larger, while relative muscle and gizzard fat mass were significantly lower compared to large birds. 12 weeks old skip-a-day fed birds largely followed the pattern of 4 weeks old small birds. In the present study, no clear signs of poorer welfare in small broiler breeders could be seen and the morphometric differences might suggest different ways to cope with feed competition. A larger gastrointestinal tract might indicate long-term investments and maybe that smaller broiler breeders, and skip-a-day fed birds, are better habituated to feed restriction.

Keywords: chicken, broiler breeder, welfare, stress, hunger, feed restriction, skip-a-day, morphometrics.

2 Introduction

During the last decades there has been a heavy selection on juvenile growth rate, efficiency of feed conversion and breast muscle mass of meat type chickens; broilers (Mench 1988, Flock *et al*. 2005, Bessei 2006, Arnould and Leterrier 2007, Dawkins and Layton 2012). Breast muscle mass has increased with around 80 % and growth rate with over 400 % since 1957 (Zuidhof *et al*. 2014). This, together with the fact that modern broilers only need 3 kg of feed to grow to a slaughter weight of approximately 2 kg in 5 weeks (Robins and Phillips 2011), have led to an efficient chicken meat industry with low environmental impact (Renema *et al*. 2007, De Vries and De Boer 2010), but also to a wide range of welfare issues for the animals (Bessei 2006, Schmidt *et al*. 2009, Dawkins and Layton 2012, Paxton *et al*. 2014). Welfare can be defined as the individual's ability to cope with its environment (Broom 1991), were

coping refers to "the individual response to a stressor by which normally harmful physiological effects of this stressor are reduced" (Schouten and Wiepkema 1991). Poor welfare can be seen among fast growing broilers in their susceptibility to cardiovascular diseases or sudden death syndrome (Mitchell 1997, Julian 1998, Maxwell and Robertson 1998, Olkowski 2007), reduced adaptive immune function (Cheema *et al*. 2003), lameness or difficulties in walking (Kestin *et al*. 1992, Sanotra *et al*. 2001, Bradshaw *et al*. 2002, Knowles *et al*. 2008), and poor reproductive performance (Hocking 1993, De Jong and Guémené 2011). Each year, over 50 billion chickens are slaughtered for meat and the broiler industry can thereby be considered to hold some of the most severe animal welfare problems in the entire agriculture world (Dawkins and Layton 2012), which also is increasingly recognised by the consumers (De Jonge and Van Trijp 2014).

Interestingly however, welfare issues are greater in the parental stock (broiler breeders) than within the slaughter birds (De Jong and Guémené 2011). Broiler breeders are sexually mature at 20 weeks and are kept in production until an age of 60 weeks, they have a similar genetic potential for growth and growth has to be limited to avoid health problems (Katanbaf *et al*. 1989a, 1989b, Hocking *et al*. 1993, De Jong *et al*. 2002). This is usually accomplished through quantitative feed restriction during the rearing period. The restriction can be as severe as 25-35 % of *ad libitum* consumption during the most intense periods (Savory *et al.* 1993, De Jong *et al*. 2002). Although some welfare concerns are decreased when the growth rate is reduced, the feed restriction itself leads to other welfare issues in the broiler breeder industry.

One of the major issues for broiler breeder welfare is the almost unanimous evidence of chronic stress and hunger (Mench 2002, De Jong *et al.* 2002, D'Eath *et al.* 2009). Broiler breeders typically display higher plasma corticosterone concentration than *ad libitum* fed birds (De Jong *et al.* 2003), but also increased heterophil/lymphocyte (H/L) ratios (Hocking *et al.* 1993), which is argued to be a more stable indicator of long-term stress than plasma corticosterone concentration (Gross 1990, D'Eath *et al.* 2009). It has also been shown, by behavioural studies, that restricted breeders always are highly motivated to eat (De Jong *et al*. 2002, Dixon *et al.* 2014), but also that breeders on litter-based rearing systems display the same proportion of scratching and pecking for food as feral fowl, which might be considered a normal response to absence of food (Savory *et al.* 1978, Hocking *et al.* 1993).

From a poultry management perspective, feed restriction is associated with increased mass heterogeneity within flocks (Bartov *et al.* 1988, De

Beer and Coon 2007, 2009). Broilers have lost a lot of their genetic diversity as a consequence of heavy selection (Muir *et al.* 2008). Although, the heritability of body weight at 5 weeks have been estimated to 0.20-0.25, which means that there is still a considerable amount of genetic variation (Wolc *et al.* 2009, Maniatis *et al.* 2013). The large variation in body mass among broiler breeders is probably still due to competition and behavioural differences, as flocks of broiler finishers fed *ad libitum* show much more homogeneity in body mass and the large difference between the groups is the amount of feed, i.e. an environmental factor. In the industry, high flock uniformity, i.e. a low coefficient of variation (CV), is a major measurement of quality and it is highly desirable because it simplifies flock management, as for example feeding (Aviagen 2013). The birds that are weak in the competition for food, and thereby stay smaller, might be regarded as suffering from poorer welfare through more intense feed restriction and hunger. If the CV is low, the majority of the birds follow the growth trajectories set by the industry (Aviagen 2013). On the other hand, if the CV is high, there will be a higher number of birds deviating from the target mean and thereby a greater proportion of smaller birds possibly suffering from poorer welfare.

As part of the handling routines in the industry, broiler breeders are size sorted at 4 weeks of age to allow more food for the small birds and consecutively catch-up growth. To decrease the heterogeneity of flocks, different methods of feed restriction have been tested; qualitative feed restriction (where the feed is diluted) or different skip-a-day/skip-two-days methods. De Beer and Coon (2009) reported more homogenous flocks when a quantitative skip-a-day feed restriction was applied, but there are some conflicting results (Bartov *et al.* 1988). With the skip-a-day method, the amount of food on feeding days is greater and should decrease competition, but the lack of food on one or two days makes the total amount of food per week the same as for restricted every-day-fed birds. The skip-a-day method is used around the world, but in Sweden the animals have to be fed every day (SJVFS 2010:15, Ch. 1, §28), which makes the skip-a-day method illegal.

A lot of studies have focused on the differences between restricted and *ad libitum* fed breeders (e.g. Mench 1991, De Jong *et al.* 2002, 2003, De Beer *et al.* 2007, 2008), different skip-a-day regimens (e.g. Katanbaf *et al.* 1989a, 1989b, 1989c) or lines/breeds selected for high and low body weight (e.g. Dror *et al.* 1977, Nir and Nitsan 1979, Pinchasov *et al.* 1985, Katanbaf *et al.* 1988). In general, slower growth (i.e. higher feed restriction or light breeds) is reported to result in an increased baseline

plasma corticosterone concentration or H/L ratio and often an increased relative mass of the gastrointestinal tract and a decreased relative muscle and fat mass. To my knowledge, there are no previous studies on welfare differences or morphometric differences within flocks of the same age. Also, very few studies have been performed on the farm itself, which has been argued to be important to be able to apply research results on animal welfare (Dawkins 2012, Dawkins and Layton 2012).

Therefore, the present study focused on measuring the extent of hunger and stress in broiler breeder females, during the rearing phase, caused by food competition between individuals on a farm in the south of Sweden in collaboration with SweHatch AB. The naturally existing body mass variation was used to distinguish between two size groups, defined as 1-2 standard deviations under and over the mean body mass of the flock, respectively. First, the effect of hatching weight on body weight at the 4 week size sorting was quantified to be able to discard hatching weight as a main source of later growth variation. Second, body composition and stress and hunger indicators were compared between small and large 4 weeks old breeders. Third, body composition was compared between 12 weeks old breeders on different feed restriction regimens. The main hypothesis of the present study was that animals growing poorly under feed restriction would show indicators of higher levels of hunger and stress due to food competition and thereby suffering from poorer welfare. The present study also aimed to characterize morphometric differences between small and large broiler breeders as well as between birds on different feeding regimens; skip-a-day vs. every-day-fed.

3 Material & methods

3.1 Animals and management

3.1.1 On farm

The broiler breeder chickens, strain Ross 308, used in the on-farm experiments all came from a rearing farm in Bökestorp, Skåne, Sweden, which is owned by SweHatch AB. This farm got the birds as one day old chicks from Aviagen SweChick AB and kept them to an age of 20 weeks. The birds were kept in groups of about 3000 individuals in pens with an area of 240 m^2, the floor was covered with wood shavings and the ceiling height was approximately 3 m. The light cycle and temperature followed the broiler breeder manual (Aviagen 2013). Chickens were fed a broiler starter diet the first 36 days continued by a grower diet. The birds were either on an every-day-fed quantitative feed restriction regimen or a skip-a-day quantitative feed restriction regimen (same amount of food per

week but with up to two non-consecutive days without food) to follow the growth curve as described in the breeders' manual. The food was distributed automatically from a spinner mounted in the ceiling, except for the first 7 days when the food was manually distributed into the pens. Water was available *ad libitum* during the light hours from day one and gradually adjusted to achieve a feed/water ratio of 1.7-2.0 at 4 weeks of age. Data for the present study were collected from two different batches of female chickens between May and December 2014. In total there were 36000 birds on the farm at a time and the experiments affected about 3000 birds in total. The number of experimental animals is large because all birds in a pen had to have the same feeding regimen. 124 birds were blood sampled and/or sacrificed for dissection. All experiments and the procedures were approved by Malmö-Lund's ethical committee (Dnr. M 71-14).

3.1.2 In lab

The lab experiment was performed in an approved chicken facility at Linköping University, Sweden. 40 one day old Ross 308 broilers were bought from SweHatch AB and kept in a 2 m^2 cage in the lab. The cage floor was covered with wood shavings and a heat lamp was installed for the first week to get a temperature at animal level of 30-33 °C. The temperature was then decreased to room temperature, approximately 20 °C. The light was on between 8:30 and 17:30. A broiler starter feed was given with a quantitative feed restriction regimen to follow the growth curve as described in the breeder's manual (Aviagen 2013) and water was offered *ad libitum*. The experiments took place in September-October 2014 and all the procedures were approved by Malmö-Lund's ethical committee (Dnr. M 71-14).

3.2 Data collection

3.2.1 Blood sampling and processing

Blood samples were obtained from the ulnar vein on the right wing with an EDTA (0.5 M in H_2O) coated needle and syringe within 2 minutes of capture of the birds. 8 µl drops were used to produce two blood smears per bird on glass slides to determine heterophil/lymphocyte ratio (H/L ratio). Glucose values were obtained with a point-of-care clinical glucose meter (Accu-Chek Aviva). On a later date, the blood was analysed for corticosterone concentration by the use of a corticosterone ELISA kit (ADI-900-097, Enzo). The blood samples were stored in 1.5 ml Eppendorf tubes on ice and within 30 minutes they were centrifuged for about 5 minutes in a tabletop centrifuge. The plasma was transferred to

0.2 ml PCR tubes and frozen immediately in a cryoshipper (CXR100, Taylor-Wharton) for transportation before being stored in a -80 °C freezer at Linköping University. After blood sampling the birds were weighed with an accuracy of 1 g by the use of a digital scale and sacrificed by decapitation. Data collection took place during the chickens' dark hours (3:30 p.m. – 1:00 a.m.).

3.2.2 Heterophil/lymphocyte ratio

Blood smears were fixated in methanol for 30 seconds, stained with Giemsa (Histolab Products AB) for 30 minutes and carefully washed with water. 100 cells per blood smear were counted and classified into heterophils, lymphocytes, eosinophils, basophils and monocytes in a light microscope with 100x magnification. All samples were analysed by the same person and the counting was blinded. To obtain the H/L ration the number of heterophils were divided by the number of lymphocytes and mean values of the duplicates were calculated.

3.2.3 Dissections

The carcasses were thawed and dissected to determine organ masses and intestine lengths. Masses were obtained with the accuracy of 0.01 g by the use of a digital scale for ventricles, liver, spleen, gizzard fat, crop and stomach content (food remaining in the crop, proventriculus and gizzard), crop, proventriculus, gizzard, pancreas, left lung, left kidney, bursa of Fabricius and the muscles pectoralis major, iliotibialis, and gastrocnemius. Intestine lengths were obtained with the accuracy of 1 mm by the use of a ruler for small intestine (divided into duodenum, jejunum and ileum), large intestine and mean caeca.

3.2.4 Circadian rhythm of glucose

40 broilers on an every-day-fed quantitative feed restriction regimen were blood sampled at 4 weeks of age in the course of a week to obtain the circadian rhythm of glucose. In total, 12 birds were sampled every second hour both day and night and the glucose concentration was measured with a point-of-care clinical glucose meter (Accu-Chek Aviva). It was not possible to avoid resampling of the birds, but a schedule was created to avoid resampling too early. The measurements were treated as if they were independent.

3.3 Statistical analysis

To test for differences between two groups (small and large), permutations tests (see appendix 1), which has been argued to be superior to *t* and *F*-tests in biomedical research (Ludbrook and Dudley 1998), were performed in the program StatBoss Permutations tester 1.0. For multiple comparisons, a cluster analysis which builds on correlations was performed in Minitab 17 (see appendix 2) and the number of clusters with a similarity above 50 % was then used to make a Bonferroni correction of the level of significance. Correlations were investigated using Pearson's correlation tests. One-way ANOVAs and Tukey's multiple comparison tests were performed to find differences in glucose concentration on different time points and to find differences in body size between four groups of the 12 week old chickens. Two-way ANOVAs and Bonferroni posthoc tests were performed to test for differences between two groups (small and large) of two different treatments (SKIP and control). The program GraphPad Prism 5 was used to perform ANOVAs and correlation tests, as well as for creating graphs. Differences were regarded as statistically significant if $P \leq 0.05$. Values are shown as mean (SD).

4 Results

4.1 Size at hatching only explains 8 % of the variation observed at 4 weeks of age

422 female broiler breeder chicks were weighed and their tarsometatarsal length measured at 1 day of age. They were marked with numbered aluminium wing tags and recaptured and measured again at 4 weeks of age. Body mass and tarsometatarsal length at 4 weeks correlated significantly with the corresponding values at hatching as shown in figure 1. The coefficient of determination (r^2) was in both cases rather weak, indicating that size at hatch only explained about 8 % of the size variation in broiler breeder females at 4 weeks of age (fig 1).

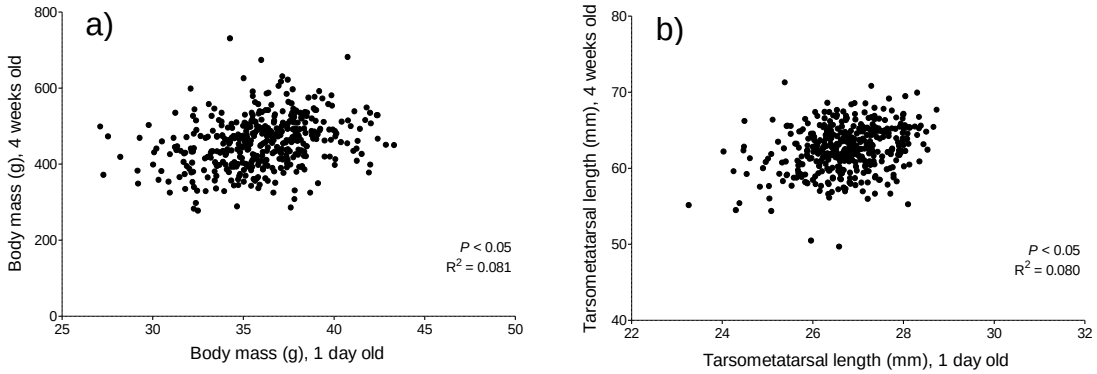

Figure 1. Correlations between 1 day and 4 weeks (a) body mass and (b) tarsometatarsal length of broiler breeder females. Pearson's correlation tests, n = 422.

4.2 No clear signs of elevated hunger or stress levels in small broiler breeders

At 4 weeks of age, the H/L ratio was significantly higher in large birds (Permutations test (see appendix 1), $P = 0.0293$, fig 2a), which might indicate a higher concentration of circulating corticosterone (Gross and Siegel 1983). Although, no difference in plasma corticosterone concentration between the two size groups could be observed ($P = 0.9298$, fig 2b).

The concentration of glucose in the blood did not differ between the groups ($P = 0.9810$, fig 2c), but there was a significant negative time effect on the glucose concentration ($r^2 = 0.184$, $P = 0.0057$, fig 3). The in lab experiment revealed that the concentration of plasma glucose varies between the light and dark hours of the day (fig 4). This diurnal rhythm is evident in both size groups, but might have made it difficult to compare them as the variation increased.

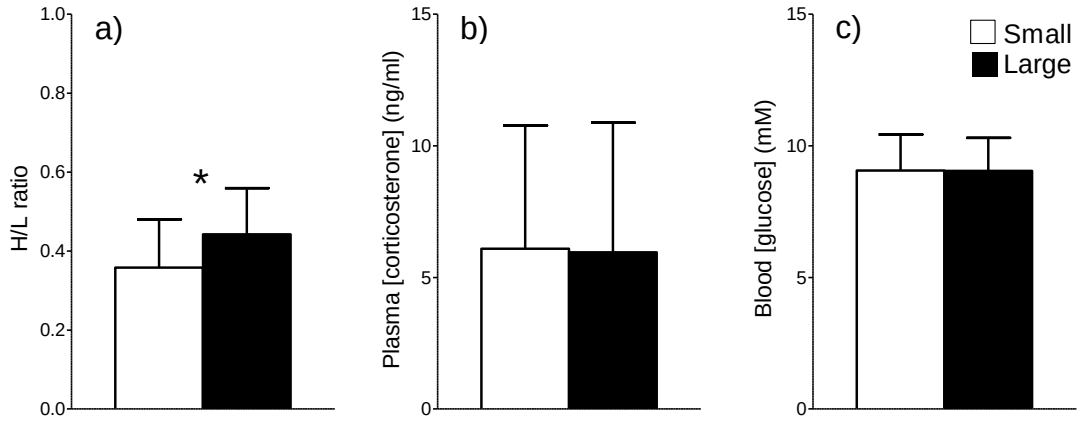

Figure 2. Comparison of mean and standard deviations of (a) heterophil/lymphocyte ratio (H/L ratio), (b) plasma corticosterone concentration and (c) blood glucose concentration between small and large 4 weeks old broiler breeder females. Permutations tests, small n = 21, large n = 21. Asterisks indicate significant differences (P ≤ 0.05).*

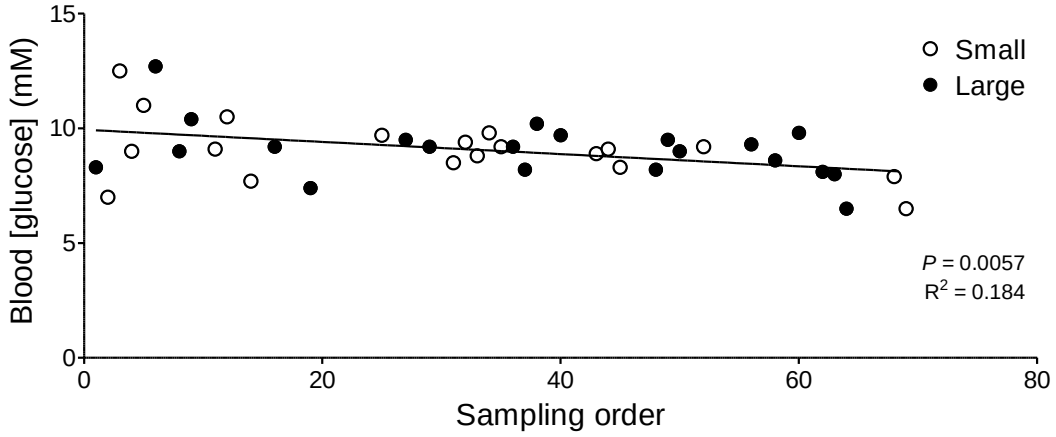

Figure 3. Correlation between blood glucose concentration and time (sampling order) in 4 weeks old broiler breeder females. Pearson's correlation test, small n = 21, large n = 19.

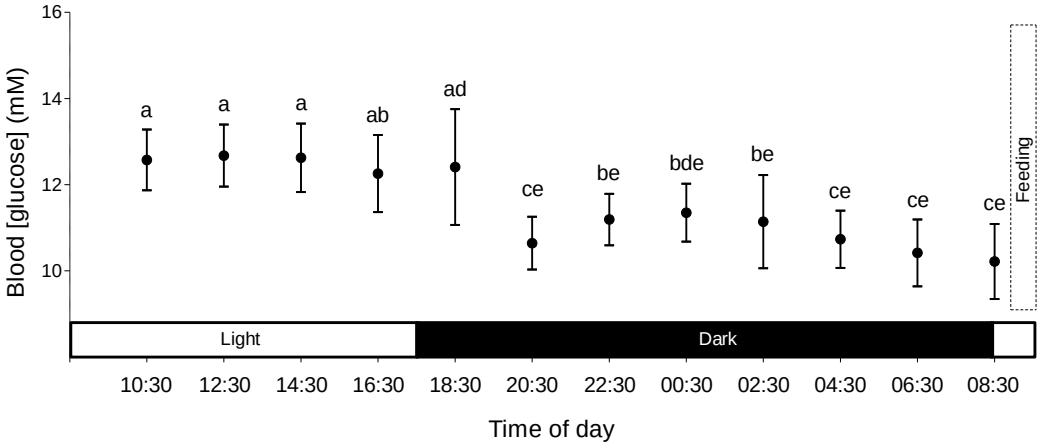

Figure 4. Means and standard deviations of blood glucose concentration on different time points after feeding in 4 weeks old broilers. One-way ANOVA and Tukey's multiple comparison tests, n = 12. Values that do not share a common superscript are significantly different at P ≤ 0.05.

4.3 Small broiler breeders have a relatively heavier GI tract and lighter muscles

At 4 weeks of age, 36 small and 33 large broiler breeder females were sacrificed and dissected to determine organ masses and intestine lengths. Small birds had a significantly heavier proventriculus ($P < 0.0001$), gizzard ($P = 0.0003$) and pancreas ($P = 0.0001$) as well as longer intestines ($P < 0.0001$) relative to lean body mass (body mass without remaining food content and gizzard fat) compared to large birds (fig 5). When comparing the intestine lengths relative to tarsometatarsal length, there were no differences between the size groups (all $P > 0.1$, fig 6). Relative mass of the liver ($P < 0.0001$) were significantly higher in small birds (table 1), but there were no differences between groups in the relative mass of lung ($P = 0.5569$), kidney ($P = 0.5045$), spleen ($P = 0.7925$), and Bursa of Fabricius ($P = 0.0848$, table 1). The relative masses of the crop ($P = 0.0293$) and ventricles ($P = 0.0313$) were larger in small birds only before the Bonferroni correction. Small birds had significantly more food left in the upper gastrointestinal tract (crop, proventriculus and gizzard, $P = 0.0015$) relative to lean body mass (table 1), but there was no difference in absolute terms ($P = 0.4370$). Large birds tended to have larger relative fat deposits ($P = 0.0498$, table 1) and *pectoralis major* ($P = 0.0146$) and had significantly larger relative mass of *iliotibialis* ($P = 0.0039$) and *gastrocnemius* muscles ($P = 0.0032$, in absolute terms all $P = 0.0001$, fig 7).

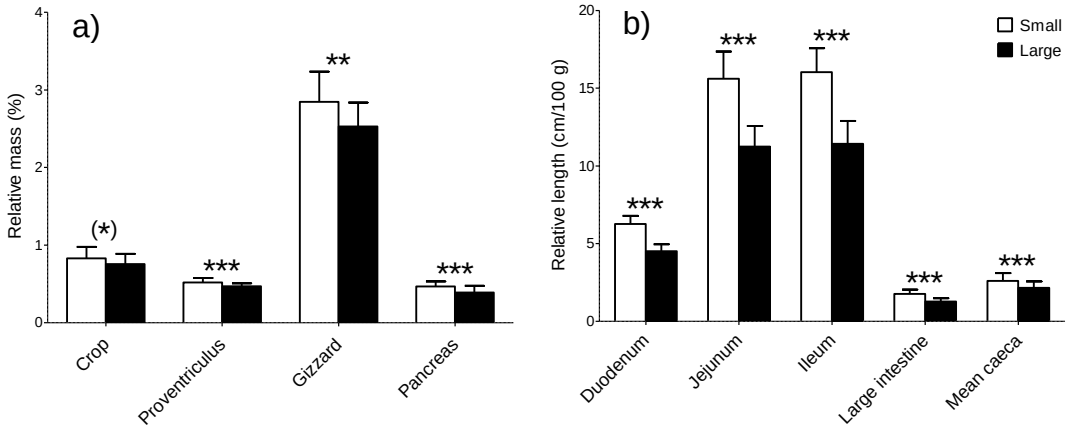

Figure 5. Comparison of (a) mean (SD) organ masses and (b) mean (SD) intestine lengths relative to lean body mass between small and large 4 weeks old broiler breeder females. Permutations tests, small n = 36, large n = 33. Bonferroni corrected for multiple comparisons. Asterisks indicate significant differences (P ≤ 0.0125, ** P ≤ 0.0025, *** P ≤ 0.00025, (*) P ≤ 0.05 without Bonferroni correction).*

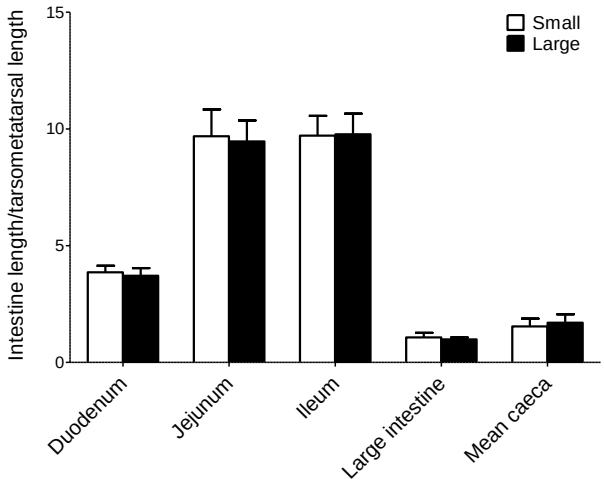

Figure 6. Comparison of mean (SD) intestine lengths relative to tarsometatarsal length between small and large 4 weeks old broiler breeder females. Permutations tests, b) small n = 15, large n = 12.

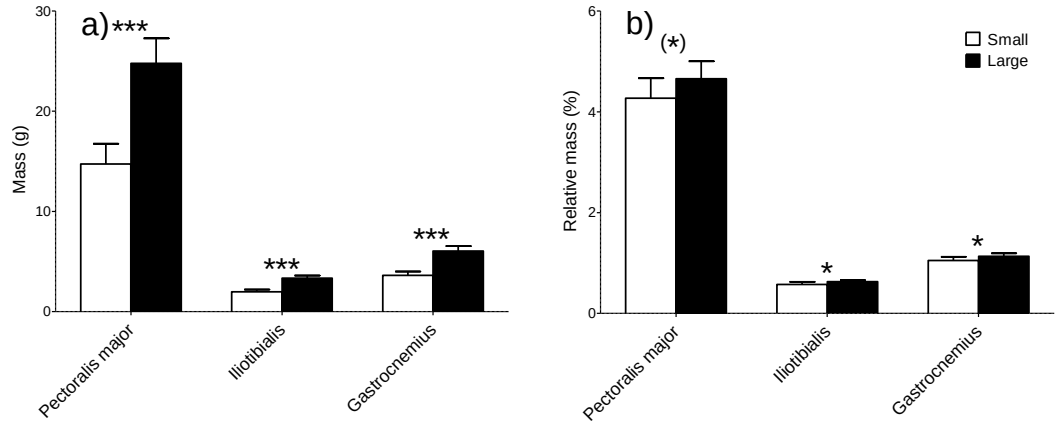

Figure 7. Comparison of (a) mean (SD) absolute muscle mass and (b) mean (SD) muscle mass relative to lean body mass between small and large 4 weeks old broiler breeder females. Permutations tests, small n = 15, large n = 12. Bonferroni corrected for multiple comparisons. Asterisks indicate significant differences (P ≤ 0.0125, ** P ≤ 0.0025, *** P ≤ 0.00025, (*) P ≤ 0.05 without Bonferroni correction).*

Table 1. Comparison of body size, organ masses relative to lean body mass, fat depots and crop and stomach content between small and large 4 weeks old broiler breeder females. Permutations tests, small n = 36, large n = 33, values are shown as mean (SD). Bonferroni corrected for multiple comparisons. Asterisks indicate significant differences within a row (P ≤ 0.0125, ** P ≤ 0.0025, *** P ≤ 0.00025).*

Item	Small	Large	*P*
Lean body mass (g)	394 (48)	570 (36)***	< 0.0001
Tarsometatarsal length (mm)[1]	58.34 (1.61)	66.00 (1.44)***	< 0.0001
Relative ventricles mass (%)	0.41 (0.07)	0.37 (0.05)	0.0313
Relative liver mass (%)	3.04 (0.29)	2.74 (0.26)***	< 0.0001
Relative left lung mass (%)	0.32 (0.06)	0.32 (0.06)	0.5569
Relative left kidney mass (%)	0.32 (0.05)	0.31 (0.04)	0.5045
Relative spleen mass (%)	0.11 (0.03)	0.11 (0.03)	0.7925
Relative bursa of Fabricius mass (%)[2]	0.25 (0.07)	0.27 (0.06)	0.0848
Crop and stomach content (g)	23.26 (14.13)	20.89 (10.61)	0.4370
Relative crop and stomach content (%)	5.91 (3.44)	3.70 (1.90)**	0.0015
Gizzard fat (g)	0.69 (0.38)	1.35 (1.03)***	< 0.0001
Relative gizzard fat (%)	0.17 (0.09)	0.23 (0.17)	0.0498

[1] Small n = 15, large n = 12.
[2] Small n = 24, large n = 17.

4.4 Broiler breeders on a skip-a-day feeding schedule have heavier livers and lighter muscles

At 12 weeks of age, 29 broiler breeder females on a skip-a-day feeding schedule (15 small and 14 large) and 26 control birds on an every-day-fed schedule (14 small and 12 large) were sacrificed and dissected to determine organ masses and intestine lengths.

Because the birds were selected for sampling based on their body mass there was a significant difference between the small and large groups ($F = 243.5$, $P < 0.001$) but not between skip-a-day and controls ($F = 3.126$, $P = 0.083$, table 2). Control large birds, however, had a significantly longer tarsometatarsal length compared to SKIP large birds ($F = 9.579$, $P = 0.003$, table 2).

Effects of feeding regimen could be seen for both size groups at 12 weeks of age (table 3-4). SKIP birds of both size groups had a significantly heavier liver compared to control birds, both in absolute ($F = 97.43$, $P < 0.001$) and relative terms ($F = 119.2$, $P < 0.001$, fig 8). SKIP large birds also had significantly heavier ventricles and left lung, absolute and relative, compared to control large birds (fig 8).

Some effects of size group on body composition could also be seen among the 12 week old birds (table 3-4). In absolute terms, SKIP large birds had significantly heavier ventricles, liver and left lung compared to SKIP small birds (fig 8a). In relative terms, control small birds had significantly heavier ventricles and left lung compared to control large, while the opposite pattern was seen on left lung within SKIP birds (fig 8b, table 3-4).

Table 2. Body size of 12 weeks old female broiler breeder chickens of two size groups on two different feeding regimens. One-way ANOVA and Tukey's multiple comparison tests.

	SKIP S n = 15	**Control S** n = 14	**SKIP L** n = 14	**Control L** n = 12
Lean body mass (g)	1168 (71)[a]	1185 (58)[a]	1409 (58)[b]	1449 (48)[b]
Tarsometatarsal length (mm)	94.56 (1.74)[a]	95.25 (3.13)[a]	98.39 (1.56)[b]	101.45 (2.22)[c]

[a-c] Values within a row lacking a common superscript differ (P < 0.05).

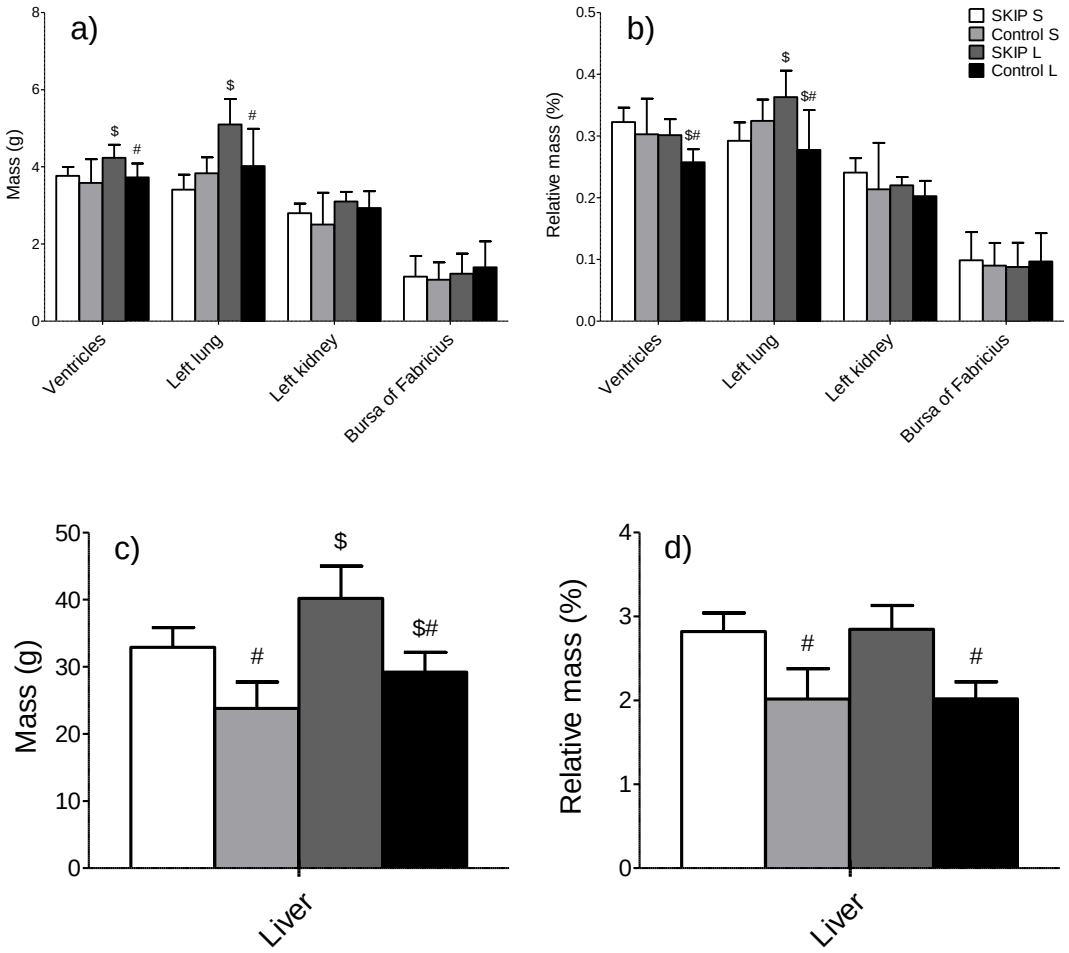

Figure 8. Comparison of (a, c) mean (SD) absolute organ masses and (b, d) mean (SD) organ masses relative to lean body mass between two groups (small (S), large (L)) of 12 weeks old broiler breeder females on two different feeding regimens (Skip-a-day (SKIP), every day fed (control)). Two-way ANOVAs and Bonferroni posthoc tests, SKIP S n = 15, control S n = 14, SKIP L n = 14, control L n = 12.

[#] Control is significantly different from SKIP within a size group ($P < 0.05$).

[$] L is significantly different from S within a feeding regimen ($P < 0.05$).

In both absolute and relative terms, control small birds had significantly heavier muscles compared to SKIP small and gastrocnemius was significantly heavier in control large birds compared to SKIP large (fig 9, table 3-4).

All muscles were significantly heavier in absolute terms for large birds, both within SKIP and control (fig 9a). The size groups differed within feeding regimen on the relative pectoralis major mass and SKIP large birds had a relatively heavier iliotibialis compared to SKIP small (fig 9, table 3-4).

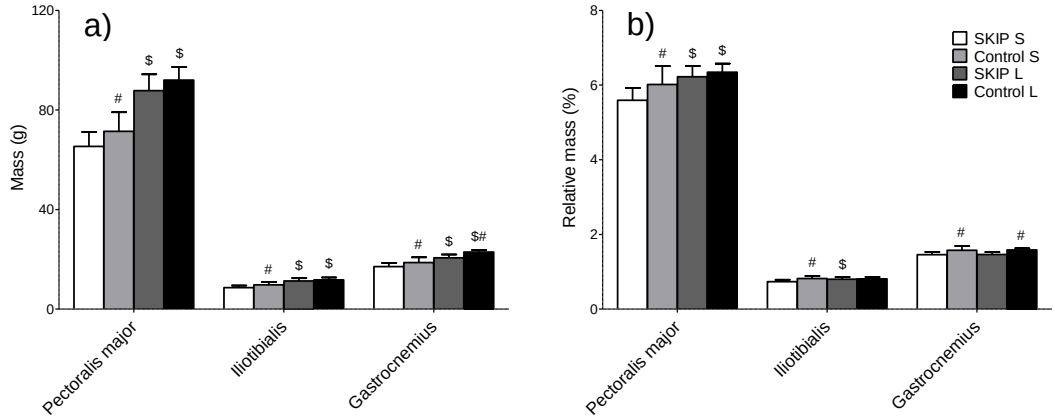

Figure 9. Comparison of (a) mean (SD) absolute muscle masses and (b) mean (SD) muscle masses relative to lean body mass between two groups (small (S), large (L)) of 12 weeks old broiler breeder females on two different feeding regimens (Skip-a-day (SKIP), every day fed (control)). Two-way ANOVAs and Bonferroni posthoc tests, SKIP S n = 15, control S n = 14, SKIP L n = 14, Control L n = 12.

[#] Control is significantly different from SKIP within a size group ($P < 0.05$).

[$] L is significantly different from S within a feeding regimen ($P < 0.05$).

Table 3. Two-way ANOVA table of absolute organ and muscle masses of 55 broiler breeder females at 12 weeks of age. Skip-a-day fed small n = 15, skip-a-day fed large n = 14, every-day-fed small n = 14, every-day-fed large n = 12. Asterisks indicate significant differences (P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001).*

Source	*df*	*MS*	*F*	*P*
Ventricles				
Size group	1	1.276	7.594	0.008**
Feeding regimen	1	1.647	9.806	0.003**
Size group X Feeding regimen	1	0.3649	2.172	0.147
Liver				
Size group	1	548.5	38.92	< 0.001***
Feeding regimen	1	1373	97.43	< 0.001***
Size group X Feeding regimen	1	12.07	0.8567	0.359
Left lung				
Size group	1	11.99	30.11	< 0.001***
Feeding regimen	1	1.469	3.687	0.060
Size group X Feeding regimen	1	7.684	19.29	< 0.001***
Left kidney				
Size group	1	1.839	7.441	0.009**
Feeding regimen	1	0.7327	2.965	0.091
Size group X Feeding regimen	1	0.05027	0.2034	0.654
Bursa of Fabricius				
Size group	1	0.5442	1.844	0.180
Feeding regimen	1	0.02066	0.07003	0.792
Size group X Feeding regimen	1	0.2079	0.7046	0.405
Left pectoralis major				
Size group	1	6324	151.1	< 0.001***
Feeding regimen	1	356.9	8.529	0.005**
Size group X Feeding regimen	1	11.77	0.2812	0.598
Left iliotibialis				
Size group	1	76.21	73.33	< 0.001***
Feeding regimen	1	8.493	8.172	0.006**
Size group X Feeding regimen	1	1.594	1.534	0.221
Left gastrocnemius				
Size group	1	204.2	88.67	< 0.001***
Feeding regimen	1	51.93	22.55	< 0.001***
Size group X Feeding regimen	1	1.443	0.6266	0.432

Table 4. Two-way ANOVA table of relative organ and muscle masses of 55 broiler breeder females at 12 weeks of age. Skip-a-day fed small n = 15, skip-a-day fed large n = 14, every-day-fed small = 14, every-day-fed large = 12. Asterisks indicate significant differences (P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001).*

Source	*df*	*MS*	*F*	*P*
Relative ventricles				
Size group	1	0.01514	12	0.001***
Feeding regimen	1	0.01387	10.99	0.002**
Size group X Feeding regimen	1	0.001986	1.574	0.215
Relative liver				
Size group	1	0.003103	0.04056	0.841
Feeding regimen	1	9.119	119.2	< 0.001***
Size group X Feeding regimen	1	0.0022	0.02876	0.866
Relative left lung				
Size group	1	0.001979	1.027	0.316
Feeding regimen	1	0.009618	4.99	0.030*
Size group X Feeding regimen	1	0.04726	24.52	< 0.001***
Relative left kidney				
Size group	1	0.00344	1.94	0.170
Feeding regimen	1	0.006791	3.829	0.056
Size group X Feeding regimen	1	0.0003144	0.1773	0.676
Relative bursa of Fabricius				
Size group	1	0.00005861	0.03333	0.856
Feeding regimen	1	6.968E-08	4E-05	0.995
Size group X Feeding regimen	1	0.001043	0.5931	0.445
Relative left pectoralis major				
Size group	1	3.147	25.38	< 0.001***
Feeding regimen	1	1.007	8.118	0.006**
Size group X Feeding regimen	1	0.3109	2.507	0.120
Relative left iliotibialis				
Size group	1	0.01174	3.317	0.074
Feeding regimen	1	0.0304	8.587	0.005**
Size group X Feeding regimen	1	0.019	5.317	0.025*
Relative left gastrocnemius				
Size group	1	0.0003431	0.05126	0.822
Feeding regimen	1	0.1875	28.01	< 0.001***
Size group X Feeding regimen	1	0.00006915	0.01033	0.919

5 Discussion

The body size at hatching could only explain about 8 % of the body size variation at 4 weeks of age (Fig 1) and could thereby be discarded as a major factor of the later variation. Large birds showed a higher H/L ratio (Fig 2a), which is related to higher concentration of circulating corticosterone and indicates a higher stress level. Small birds had relatively heavier stomachs, pancreas and liver (Fig 5, Table 1), while large birds had relatively heavier muscles (Fig 7). The intestine lengths relative to body mass were greater in small birds (Fig 5), but there were no differences when comparing to tarsometatarsal length (Fig 6). There was in general no clear evidence of poorer welfare among small birds, at least from two of the variables commonly used as stress indicators. Birds on a skip-a-day diet had a relatively heavier liver (Fig 8) and, in general, lighter muscles (Fig 9).

As expected, size at hatching had a significant, but low effect on size later in life (Fig 1) and could be discarded as a major factor of later growth variation. The same result was given in a pilot lab experiment ($r^2 = 0.08$, n = 108, data not published), were the farm conditions were imitated as closely as possible. Originally, the chickens from the second batch in the present study were divided in four groups dependent on hatching weight and 4 week weight; small-small, small-large, large-small, and large-large. It was not possible to get a large enough data set of birds catching up or falling behind into the strict ±1-2 SD groups at 4 weeks, but the small-large group consistently followed the results of the large-large group and large-small followed small-small. This indicates that the body weight at 4 weeks had a greater effect, at least on body composition, than did the hatching weight. It also means that the size variation at 4 weeks is more affected by other factors as competition and/or behavioural differences than purely the hatching mass.

At 4 weeks of age, large birds displayed a higher H/L ratio and this might indicate a higher stress level (Fig 2a). The corticosterone concentration did not differ between the groups (Fig 2b), but the values could not be regarded as baseline values. Even though the birds were blood sampled within the time it takes for them to react to a stressor with elevated corticosterone concentration (2 minutes, Chloupek *et al.* 2011), the birds were obviously affected by the experimenters' presence in their pen and their values were more similar to those reported at 15 minutes after a presented stressor (Cockrem 2007). Gross and Siegel (1983) reported an increased H/L ratio when injecting corticosterone and thereby proved a connection between the two, although it has been argued that H/L ratio is a better measure of long term stress and as a welfare indicator (Gross and

Siegel 1983, McFarlane and Curtis 1989). The problems mentioned with corticosterone as a long term stress and welfare indicator are mainly its variation over short time periods (Gross and Siegel 1983, De Jong *et al.* 1992) and its close connection to fasting (Mench 1991, Hocking *et al.* 1993, D'Eath *et al.* 2009). It has also been shown that refeeding of fasted chicks result in a corticosterone concentration decrease proportional to the amount of feed (Harvey and Klandorf 1983) and even that only the visual stimuli of food have about the same effect (Klandorf and Harvey 1984). Therefore, the commonly reported higher baseline plasma corticosterone concentration in restricted broiler breeders (Mench 1991, De Jong *et al.* 2002) might actually represent predictable physiological responses to fasting (Hocking *et al.* 1993). H/L ratio should be at least a better factor as it does not suffer from the problems of fast direct changes in responses to feeding (D'eath *et al.* 2009). It is still connected to corticosterone concentration, however, and there are some confusing results reported when comparing different feeding regimens (reviewed by D'eath *et al.* 2009). From these two variables there was no clear evidence of poorer welfare in small broiler breeders.

The blood glucose levels did not differ between the groups (Fig 2c) and that might indicate that they had been without food for approximately the same amount of time, i.e. the small birds had maybe not been eating less food and thereby not emptied their crops earlier. Glucose concentration decreased with time in the on-farm experiment (Fig 3), but in lab there was a more abrupt decrease followed by a steady lower value (Fig 4). It seems plausible from the present results that a diurnal rhythm in glucose concentration might be present in feed restricted broiler breeders, even though there are some conflicting results from *ad libitum* fed broilers (Twiest and Smith 1970 vs Raheja 1973).

The most striking differences in body composition were the relatively heavier stomachs, liver and pancreas, and lighter muscles of small birds (Fig 5, 7). There were no differences in absolute mass of remaining food in the upper gastrointestinal tract, but large birds tended to have relatively more fat surrounding the gizzard (Table 1). The relative lengths of the intestines did not differ between the groups when making the comparison to tarsometatarsal length (Fig 6). When making the comparison of intestine lengths to lean body mass though, as previously done by for example Katanbaf and colleagues (1989c), small birds had relatively longer intestines, even though this should be regarded as a dimensionally incorrect comparison (Fig 5). Altogether, it seems like small and large birds prioritised different growth parameters. Large broiler breeders might have gone for the immediate advantages by investing in rapid

muscle growth and won in the competition for food simply because they were larger, while small birds might have gone for more long term advantages by investing in the gastrointestinal tract and getting a more efficient digestion system. It is possible that which strategy to go for is decided already during the chicks' first week in life. If we hypothesize that small broiler breeders gets less food the first week, maybe due to coping styles (Koolhaas *et al.* 1999), then they may respond to the low-food environment (developmental plasticity, Kotrschal *et al.* 2014) by making their digestion system more efficient through a larger gastrointestinal tract. It has been shown that animals in low-food environments are more efficient in using energy (Kotrschal *et al.* 2014) and that the gastrointestinal tract undergoes rapid growth during the first week of life (Lilja 1983, Katanbaf *et al.* 1988, Noy and Sklan 1998). Many studies have focused on differences between restricted and *ad libitum* fed broiler breeders (e.g. Mench 1991, De Jong *et al.* 2002, 2003, De Beer *et al.* 2007, 2008) and in general, they find relatively larger gastrointestinal tract, pancreas and liver in restricted birds, while the *ad libitum* fed ones typically have more abdominal fat and larger muscles. These results would also be in line with the results of the present study, if the hypothesis of less food for small birds the first week is true and they thereby can be regarded as more feed restricted. Differences in body composition of skip-a-day fed birds compared to every-day-fed birds largely followed the same relationship as for small compared to large (Table 3-4). Relative liver mass of skip-a-day fed birds was significantly larger (Fig 8), while relative muscles mass in general were lower (Fig 9). The rest of the gastrointestinal tract also in general followed the same pattern (Anouschka Middelkoop 2015, personal communication).

In conclusion, the original hypothesis of poorer welfare of small birds is not supported in the present study. It seems likely that small and large broiler breeders experienced the same amount of feed restriction (no difference in absolute crop fill), that small birds and skip-a-day fed birds had a relatively larger gastrointestinal tract, pancreas and liver (a more efficient digestive system) and that small birds did not show sign of higher stress levels, which might indicate that small broiler breeders and skip-a-day fed broiler breeders were more successful in coping with feed restriction and maybe that they are better habituated to the industry environment.

5.1 Societal & ethical considerations

Broiler chickens are raised in huge numbers all around the world for lean and cheap meat. There are obvious and well documented welfare issues that are impossible to eliminate in the present breeds with an enormous

growth capacity. Animal welfare is today greatly recognised as an important subject for people in general and awareness about the present issues increases. Even though it is a long way to a problem free broiler industry, a lot can be done if the scientific world and the industry collaborate and make use of the results from research projects.

The present study can contribute with some knowledge of morphometric differences between birds of different size groups or feeding regimens and that no clear signs of welfare differences within flocks could be seen. Together, this might indicate that new ideas in the industry are needed, where they usually appreciate larger individuals rather than smaller ones. If smaller breeder females have invested more for the future, it might result in better health later in life and they actually can better suited for high egg production.

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8 Appendix

8.1 Appendix 1 – Permutations test

The permutations test is sometimes referred to as the randomisation test, rerandomisation test or exact test (Drummond and Vowler 2012). It is a statistical method to test for differences between two groups and it does not require large samples, random samples or a normally distributed population (Ludbrook and Dudley 1998, Lew 2008, Drummond and Vowler 2012). It works by randomise the data and put it in two groups in all possible arrangements independent of treatment. The *P*-value indicates the probability that the results of the experiment turned out in a certain way merely by chance (Ludbrook and Dudley 1998) and is calculated by dividing the number of possible arrangements (permutations) with a mean value equal to or more extreme than the observed by the total number of possible permutations (fig A1, Lew 2008). The test does not take in to consideration the population which the samples are taken from (which often is poorly defined in many cases, Lew 2008), but instead treat the sample as a whole population. This means that the hypothesis is much more simple and the results easier to interpret, but when the inference should be applied to (in the present study's case) other chickens it is only possible by verbal arguments (Ludbrook and Dudley 1998). As the permutations test have the same power as the Student's t-test for samples with n > 5 when the data is normally distributed and is superior for non-normally distributed data, permutations tests should be preferred when comparing two groups (Ludbrook and Dudley 1994, 1998, Lew 2008, Drummond and Vowler 2012).

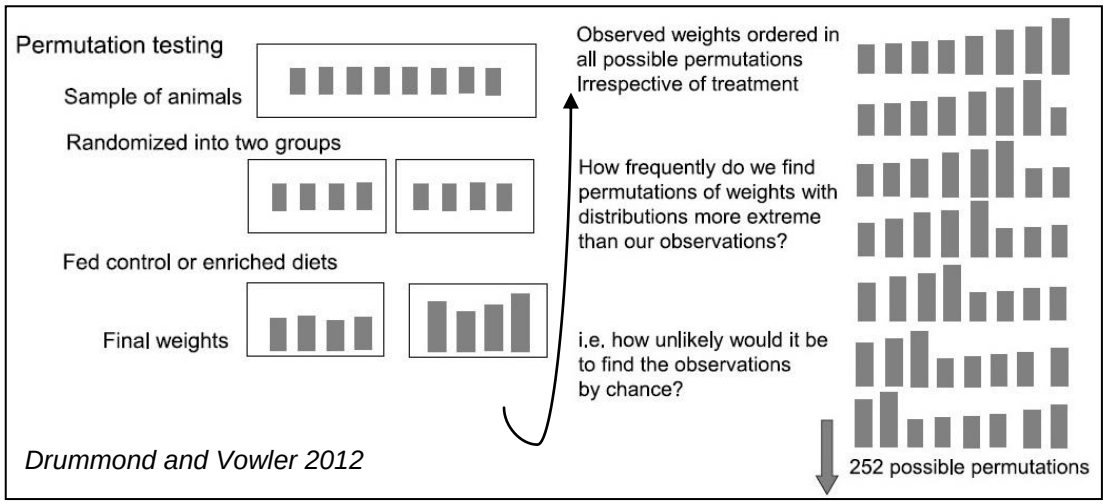

Figure A1. The permutation test assembles the observed experimental data in all possible arrangements. Each arrangement would be equally possible if the allocation of data were random. We can then assess the likelihood of the data being distributed the way they have been found to occur (Drummond and Vowler 2012).

8.2 Appendix 2 – Cluster analysis

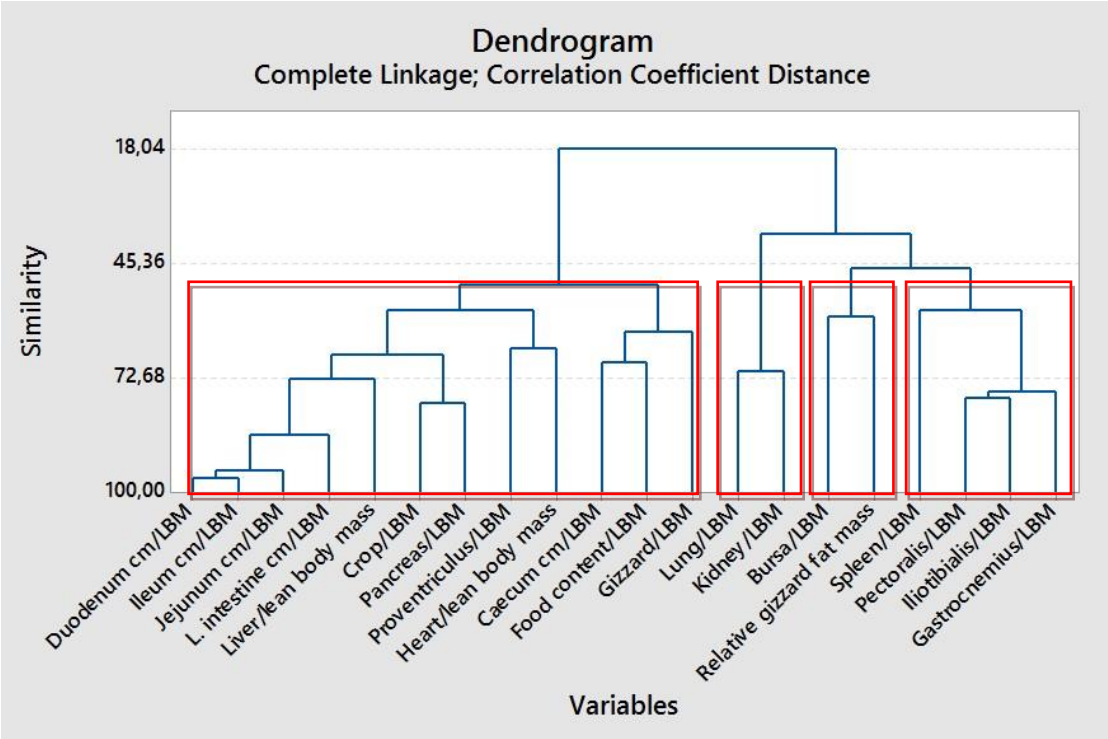

Figure A2. A cluster analysis of all relative organ masses and relative intestine lengths. The top of the red boxes are on the 50 % similarity mark and the boxes represent the four groups emerging.