Effects of Graded Dietary L-arginine Supply on Organ Growth in Four Genetically Diverse Layer Lines during Rearing Period

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Little information has been available about the influence of genetic background and dietary L-arginine (Arg) supply on organ growth of chickens. Therefore, the present study examined the effects of a graded ad libitum Arg supply providing 70, 100 and 200% of recommended Arg concentration on organ growth of female chickens from hatch to 18 weeks of age. The chickens derived from four layer lines of different phylogeny (white vs. brown) and laying performance (high vs. low). Based on residual feed and absolute body and organ weights recorded in six-week-intervals, feed consumption, changes of relative organ weights and allometric organ growth were compared between experimental groups.

Surplus Arg caused higher feed intake than insufficient Arg ($p<0.01$) that induced growth depression in turn ($p<0.05$). During the entire trial chicken’s heart, gizzard and liver decreased relatively to their body growth ($p<0.001$) and showed strong positive correlations among each other. On the contrary, proportions of pancreas and lymphoid organs increased until week 12 ($p<0.001$) and correlated positively among each other. Due to their opposite growth behaviour ($p<0.001$), internal organs were assigned to two separate groups. Furthermore, insufficient Arg induced larger proportions of bursa, gizzard and liver compared with a higher Arg supply ($p<0.05$). In contrast to less Arg containing diets, surplus Arg decreased relative spleen weights ($p<0.01$). The overall allometric evaluation of data indicated a precocious development of heart, liver, gizzard, pancreas and bursa independent of chicken’s genetic and nutritional background. However, insufficient Arg retarded the maturation of spleen and thymus compared with an adequate Arg supply.

In conclusion, the present results emphasised the essential function of Arg in layer performance, and indicated different sensitivities of internal organs rather to chicken’s dietary Arg supply than to their genetic background.

**Keywords:** allometric growth, chicken, genotype, L-arginine, organ growth, rearing

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**Introduction**

In modern egg-producing industry, chicken’s life is characterised by several marked physiological changes from hatch to the onset of laying. The rearing period can impose certain stresses to birds such as suboptimal nutritional and climatic conditions (Leeson and Summers, 1980, 1989), which influence chicken’s metabolic, endocrine and immune system as well as their production efficiency subsequently. In order to modulate these characteristics in reared chickens appropriately, specific dietary nutrients can be supplemented to the diets of chicks and pullets (Humphrey and Klasing, 2004; Tesseraud et al., 2011; Korver, 2012) such as the cationic amino acid L-arginine (Arg; Kwak et al., 1999; Wang et al., 2014b; Lieboldt et al., 2016).

In contrast to mammals, chickens are unable to synthesise Arg de novo due to a lack of urea cycle key enzymes (Tamir...
avian immune (Sung et al., 1991). NO serves as paracrine regulating mediator in the avian immune (Sung et al., 1991; Kidd et al., 2001), nervous (Gaskin et al., 2003; Wang et al., 2014a) and vascular system (Wideman et al., 1995, 1996). In addition, Arg affects the development of chicken’s lymphoid organs (Kwak et al., 2001; Deng et al., 2005) and possesses secretagogue activities by stimulating the release of several pituitary and pancreatic hormones (Barbul, 1986; Dorshkind and Horseman, 2000; Calder and Yaqoob, 2004).

With regard to the conservation of genetic resources in agriculture, Lieboldt et al. (2015) have established a chicken model consisting of four purebred layer lines differing in their phylogeny (white vs. brown) and laying performance level (high vs. low). To implement their genetically determined performance potential, high performing genotypes require larger amounts of nutrients compared to low performing ones (van der Waaij, 2004; Mirkena et al., 2010). The authors have concluded that high performing genotypes have a lower capacity to compensate unexpected environmental changes such as nutritional limitations and imbalances than low performing genotypes. The model described by Lieboldt et al. (2015) has revealed genetically dependent differences in chicken’s growth parameters, Arg utilization and requirement as well as in the susceptibility of growing chickens to dietary imbalances (Lieboldt et al., 2015, 2016). Based on these findings, we hypothesise that the growth of chicken’s internal organs responds differently to a graded Arg supply in reared chickens of four genetically diverse layer strains from hatch to 18 weeks of age.

Material and Methods

Experimental Design, Procedure and Diets
The present study was performed with 36 one-day-old female chicks of four purebred layer lines each. These strains were part of the chicken model described by Lieboldt et al. (2015), previously. Two commercial high performing genotypes (WLA and BLA) from the breeding programme of the Lohmann Tierzucht GmbH (Cuxhaven, Germany) were contrasted with two low performing ones (R11 and L68) from non-selected resource populations of the Institute of Farm Animal Genetics (Neustadt-Mariensee, Germany). Both white layer lines (WLA and R11) were of White Leghorn origin and phylogenetically closely related, but distant from BLA (Rhode Island Red) and its counterpart L68 (New Hampshire). Chicks of the present study were reared under the same conditions as described by Lieboldt et al. (2016).

After hatch chicks were equipped with wing-tags, vaccinated against Marek’s and Newcastle Disease, and distributed to diets equivalent to 70, 100 and 200% of age-specific recommended Arg supply (NRC, 1994) from hatch to week 7 and from week 8 to 18 onwards (Table 1). Consequently, the study comprised 12 experimental groups (4 genotypes x 3 diets) with 12 chicks each. The birds of each group were housed in three floor-range pens with 4 chicks each. The pens were equipped with nipple drinkers and a feeding trough for offering water and feed ad libitum. During the trial light was provided for 24 hours on days 1 and 2 and reduced to 15 hours daily in the first week of age. From week 1 to 7 daily light period was shortened in one-hour-steps weekly to 9 hours and maintained until week 18. Temperature programme followed usual specifications of chickens reared for laying.

Chicks of both age-groups were fed with a low Arg containing basal diet (LA) that was further supplemented to adequate (AA) and high Arg (HA) by adding free Arg base (crystalline, 99%, Europepta, Hannover, Germany) at the expense of corn. To ensure that Arg served as first-limiting amino acid in the basal diets of chicks and pullets, deficient L-lysine was supplemented to required levels (NRC, 1994) in these diets.

During the experiment chicks’ body weight (BW) and residual feed were recorded in six-week-intervals. At hatch and at the end of each interval one chick per pen (n=3 per group and sampling) was slaughtered after recording its BW by stunning and exsanguination through the neck vessels. After removing adherent adipose and connective tissue from eviscerated organs absolute weights of heart, liver, pancreas and gizzard without feed particles and its cuticle (koilin) on the one hand and those of the lymphoid organs bursa cloacalis, thymus and spleen on the other hand were recorded. The organ weights were presented as relative weights of BW (% of BW = [organ weight/BW]×100). Daily weight gain (DWG), daily feed intake (DFI), and the feed conversion ratio (FCR) were calculated for each six-week-interval further.

All procedures conducted in this study were in accordance with the guidelines issued by the German animal protection law and were reviewed and approved by the relevant authorities (Lower Saxony State Office for Consumer Protection and Food Safety, LAVES, Germany; 3392 42502-04-13/1186).

Analysis of Feed
The experimental diets (Table 1) were analysed for dry matter, crude ash, crude fat, crude fibre, starch, sucrose, phosphorous, calcium and Kjeldahl nitrogen (N) according to the methods of the Association of German Agricultural Analytic and Research Institutes (VDLUFA; Bassler, 1993). Crude protein of basal diets was calculated by multiplying Kjeldahl N by 6.25. Because Arg contained N twice as high as crude protein, analysed N differences between Arg supplemented diets and basal diet were multiplied by 3.13 only in order to avoid overestimation of dietary crude protein in supplemented diets. The apparent metabolisable energy con-
concentration corrected to zero N balance (AMEN) of diets was calculated according to the energy estimation equation of the World's Poultry Science Association (Vogt, 1986) further.

In order to calculate the concentrations of amino acids in the experimental diets appropriately, amino acid containing feed components other than those supplemented in their free

forms were analysed for their containing amounts of amino acids by ion exchange chromatography as described in the analytical methods of AMINODat® 4.0 (Evonik Industries, 2010).

### Modelling of Allometric Organ Growth Functions

To estimate the relationship between internal organs and...
BW in more detail, absolute organ weights were fitted regressively to the allometric growth function as proposed by Huxley and Teissier (1936) using procedure “nonlinear regression” of the software “Statistica 12.0 for the Windows™ Operating System” (Statsoft Inc., 2014). Regression coefficients $a$ and $b$ were estimated using the iterative Quasi-Newton method.

$$y(BW) = a \cdot BW^b$$

Where $y(BW)$ is chickens’ organ weight (in g) at a specific BW (in g). Regression coefficient $a$ is a constant and relates to the proportional size of the specific organ, whereas the allometric growth coefficient $b$ takes on values of smaller, equal or larger than 1 and indicates an early ($b<1$), equal ($b=1$) or late ($b>1$) organ maturation in relation to the whole body weight development (Labrèvre and Leclercq, 1994). The coefficient of determination ($R^2$) and residual standard deviation (RSD) served as criteria for goodness of fit.

**Statistical Analysis**

Statistical analysis was performed using procedure MIXED of the software package of SAS 9.4 (SAS Institute Inc., 2012, Cary, NC). The data were evaluated in a three factorial analysis of variance (ANOVA). Fixed effects were “genotype” (WLA, BLA, R11 and L68), “diet” (LA, AA and HA), and “age” (slaughtering dates at hatch and week 6, 12 and 18) as well as their two-factorial interactions. The model was formulated to account for heterogeneity of variances and degrees of freedom were estimated using the “kr” statement. Co-variance structure was modelled by a compound symmetry structure. The described model and co-variance structure were found to be most appropriate according to the Akaike Information Criterion. Effects were considered to be significant at $p \leq 0.05$ and trends were discussed at $0.05 < p < 0.1$. The Tukey-Kramer test was applied for a multiple comparison of means. Based on the described model the mean value differences were evaluated exemplarily. According to the group-specific coefficients of determination, a high proportion of variance could be explained by fitting weights of heart, liver, pancreas and gizzard as well as spleen and thymus to body weight.

Heart, liver, gizzard and bursa showed $b<1$ in each experimental group, whereas $b$ of pancreas was smaller than 1 in all groups except for HA fed BLA. Interestingly, the lymphoid organs spleen and thymus revealed stronger differences between the experimental groups. In general both organs received values of $b>1$ in BLA. However, in WLA the thymus showed $b<1$ independent of dietary Arg concentration and the spleen received values of $b<1$ when WLA was fed with adequate and surplus dietary Arg. Spleens of L68 took values of $b<1$ generally, whereas those of R11 were lower than 1 in the surplus Arg fed group only. Additionally, the thymus of both low performing genotypes showed values of $b<1$ when adequate and surplus concentrations of dietary Arg were provided.

Despite their general negative allometry ($b<1$), calculated growth curves of the bursa *cloacalis* showed that insufficiently Arg supplied chickens of white (Figure 1a) and brown (Figure 1b) genotypes tended to have larger $b$ values than adequately supplied chickens.

**Relative Organ Growth**

Relative organ weights are presented in two tables including digestive organs and heart (Table 4) as well as lymphoid organs (Table 5) from hatch to 18 weeks of age.

At hatch the heart proportion of R11 and L68 as well as the liver proportion of L68 were larger than those of the other genotypes ($p_{genotype}<0.001$; $p_{genotype*age}<0.001$). After hatch both proportions decreased continuously in all genotypes ($p_{age}<0.001$; $p_{genotype*age}<0.001$). From week 6 to 18 WLA had the highest liver proportion among genotypes.
Table 2. Effects of genotype and L-arginine supply on growth parameters from hatch to week 18

|               | LA     | AA     | HA     | LA     | AA     | HA     | LA     | AA     | HA     |
|---------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| **Body weight, g/chick** |        |        |        |        |        |        |        |        |        |
| Hatch         | 33\(^D\) | 35\(^D\) | 38\(^D\) | 37\(^D\) | 37\(^D\) | 40\(^D\) | 33\(^D\) | 34\(^D\) | 34\(^D\) |
| Week 6        | 293\(^{C, bc}\) | 368\(^{C, ab}\) | 344\(^{C, b}\) | 301\(^{C, bc}\) | 338\(^{C, b}\) | 361\(^{C, ab}\) | 268\(^{C, bc}\) | 301\(^{C, bc}\) | 250\(^{C, c}\) |
| Week 12       | 762\(^{B, d}\) | 855\(^{B, c}\) | 930\(^{B, b}\) | 645\(^{B, c}\) | 691\(^{B, e}\) | 656\(^{B, c}\) | 784\(^{B, d}\) | 872\(^{B, bc}\) | 834\(^{B, cd}\) |
| Week 18       | 957\(^{A, c}\) | 1034\(^{A, d}\) | 1104\(^{A, c}\) | 895\(^{A, f}\) | 941\(^{A, ef}\) | 1027\(^{A, d}\) | 953\(^{A, c}\) | 938\(^{A, ef}\) | 954\(^{A, e}\) |
| Daily weight gain, g/chick/d |        |        |        |        |        |        |        |        |        |
| Hatch to week 6 | 6.2\(^{B, ab}\) | 7.9\(^{B, ab}\) | 7.3\(^{B, ab}\) | 6.3\(^{B}\) | 7.2\(^{ab}\) | 7.6\(^{ab}\) | 5.6\(^{B, ab}\) | 6.4\(^{B, ab}\) | 5.1\(^{B, b}\) |
| Week 6 to 12  | 11.2\(^{A, bc}\) | 11.6\(^{A, bc}\) | 13.9\(^{A, ab}\) | 8.2\(^{d}\) | 8.4\(^{d}\) | 7.0\(^{d}\) | 12.3\(^{A, b}\) | 13.6\(^{A, ab}\) | 13.9\(^{A, ab}\) |
| Week 12 to 18 | 4.6\(^{B, b}\) | 4.3\(^{B, bc}\) | 4.1\(^{B, bc}\) | 6.0\(^{ab}\) | 6.0\(^{ab}\) | 8.8\(^{a}\) | 4.0\(^{B, bc}\) | 4.3\(^{B, bc}\) | 3.9\(^{B, bc}\) |
| Daily feed intake, g/chick/d |        |        |        |        |        |        |        |        |        |
| Hatch to week 6 | 19.6\(^{C, b}\) | 21.6\(^{C, b}\) | 22.5\(^{C, ab}\) | 22.1\(^{C, ab}\) | 20.8\(^{C, b}\) | 20.7\(^{C, b}\) | 22.3\(^{C, ab}\) | 21.2\(^{C, b}\) | 22.4\(^{C, ab}\) |
| Week 6 to 12  | 49.7\(^{B, c}\) | 53.8\(^{B, b}\) | 52.1\(^{B, bc}\) | 49.6\(^{B, c}\) | 52.0\(^{B, bc}\) | 51.98\(^{B, bc}\) | 46.1\(^{B, d}\) | 46.9\(^{B, cd}\) | 46.0\(^{B, d}\) |
| Week 12 to 18 | 68.5\(^{A, c}\) | 70.6\(^{A, c}\) | 69.6\(^{A, c}\) | 65.4\(^{A, d}\) | 65.4\(^{A, d}\) | 67.3\(^{A, cd}\) | 61.9\(^{A, e}\) | 63.7\(^{A, de}\) | 63.4\(^{A, de}\) |
| Feed conversion ratio, g/g |        |        |        |        |        |        |        |        |        |
| Hatch to week 6 | 3.16\(^B\) | 2.73\(^B\) | 3.08\(^B\) | 3.51\(^B\) | 2.89\(^B\) | 2.72\(^B\) | 3.98\(^B\) | 3.31\(^B\) | 4.39\(^B\) |
| Week 6 to 12  | 1.5\(^h\) | 2.37\(^h\) | 3.08\(^h\) | 2.89\(^h\) | 2.89\(^h\) | 2.72\(^h\) | 3.98\(^h\) | 3.31\(^h\) | 4.39\(^h\) |
| Week 12 to 18 | 4.44\(^b\) | 4.64\(^b\) | 3.75\(^b\) | 6.05\(^b\) | 6.19\(^b\) | 7.41\(^b\) | 3.75\(^b\) | 3.45\(^b\) | 3.31\(^b\) |

WLA: high performing White Leghorn; BLA: high performing Rhode Island Red; R11: low performing White Leghorn; L68: low performing New Hampshire; LA, AA, HA: low, adequate and high L-arginine supplied diets; PSEM: pooled standard error of means; GT: genotype

A–D: LSMeans values with PSEM (n=3 chicks/group) of one trait in the same column without common superscripts differ significantly (p<0.05)

a–f: LSMeans values with PSEM (n=3 chicks/group) in the same row without common superscripts differ significantly (p<0.05)
(p_{genotype}<0.001; p_{genotype*age}<0.001), while L68 showed the highest heart proportion from week 6 to 12. Differences of heart proportions disappeared between groups until week 18. Whereas the relative heart weight was not affected by dietary Arg (pdiet=0.704), LA caused higher liver proportions than AA and HA (pdiet<0.01).

Furthermore, high performing genotypes showed higher relative gizzard weights than low performing ones at hatch (p_{genotype*age}<0.001). Afterwards white genotypes exhibited larger gizzard proportions than BLA (p<0.001), which decreased continuously until the end of trial (p_{age}<0.001; p_{genotype*age}<0.001; p_{diet*age}<0.005). However, gizzard proportions of brown genotypes decreased until week 12 only, remained constant and differed significantly from white genotypes at week 18 (p<0.001). In contrast to AA, LA lowered the gizzard proportion of BLA significantly. However, lower gizzard proportions were induced by HA in R11 and WLA and by AA in R11 additionally (p<0.001).

Moreover, R11 showed the lowest pancreas proportion among genotypes (p_{genotype}<0.05). After hatch relative pancreas weight increased in genotypes except for L68, peaked at week 6 and decreased slightly until the end of trial (p_{age}<0.001; p_{genotype*age}<0.001). On the contrary, L68 achieved its lowest pancreas proportion at week 12 already and remained constant. From hatch to week 6 L68 and WLA showed larger pancreas proportions than R11 and BLA (p<0.001), but group differences disappeared until week 18. AA even tended to cause larger pancreas proportion than LA (p_{diet}=0.076).

In general, bursa and thymus proportions of WLA and L68
were larger than those of R11 and BLA ($p_{\text{genotype}} < 0.001$; $p_{\text{genotype} \times \text{age}} < 0.001$). From hatch to week 6 relative bursa weight of all genotypes and that of WLA thymus increased, remained constant until week 12 and decreased afterwards ($p_{\text{age}} < 0.001$; $p_{\text{genotype} \times \text{age}} < 0.001$). However, R11 did not differ in relative thymus weight until week 6, increased in the following 6 weeks and decreased thereafter. In brown chickens thymus proportions decreased already from week 6 to 12 and remained constant until week 18 ($p < 0.001$). LA tended to induce larger bursa proportions than both other diets ($p_{\text{diet}} = 0.052$). On the contrary, AA and HA tended to induce higher relative thymus weights in WLA than LA ($p_{\text{genotype} \times \text{diet}} = 0.061$). From week 6 to 18 thymus proportions remained constant in LA and HA fed chickens ($p < 0.01$), while AA caused larger proportions than LA at week 6. At week 18 this relation became conversely ($p_{\text{diet} \times \text{age}} < 0.05$).

Finally, highest and lowest spleen proportions were found in low performing chickens ($p_{\text{genotype}} < 0.001$; $p_{\text{genotype} \times \text{diet}} < 0.05$). At hatch R11 had a smaller spleen proportion than the other genotypes ($p < 0.001$). The relative spleen weight increased until week 6 in WLA and until week 12 in R11, and both decreased afterwards ($p_{\text{age}} < 0.001$; $p_{\text{genotype} \times \text{age}} < 0.001$). From hatch to week 6 spleen proportions of brown genotypes increased, remained constant until week 12 and decreased in the following ($p < 0.001$). From week 6 to 12 L68 showed higher relative spleen weights than high performing genotypes ($p < 0.001$), whereas LA and AA caused larger spleen proportions than HA generally ($p_{\text{diet}} < 0.01$).

In addition to the analysis of variance, the relative weights of internal organs were correlated with each other forming organ groups of similar growth behaviour. Strong positive correlations were found between the relative weights of heart, liver and gizzard on the one hand (Pearson correlation coefficient: 0.749, $p < 0.001$) and the pancreas and lymphoid organs bursa, thymus and spleen on the other hand (Pearson correlation coefficient: 0.476, $p < 0.001$). Relative weights of lymphoid organs were positively correlated with each other (Pearson correlation coefficient: 0.527, $p < 0.001$). Interestingly, the relative weights of metabolically important organs heart, liver and gizzard were negatively correlated with those of bursa and spleen (Pearson correlation coefficient: 0.422, $p < 0.001$).

**Discussion**

Optimal growth in chickens is based on the complex interaction of metabolic, endocrine and immunological mechanisms (Scanes, 2009). Thereby the protein and amino acid metabolism forms one of the pivotal pillars of growth (Bequette, 2003). In addition to their proteinogenic functions, amino acids such as Arg serve as signal mediators (reviewed in: Tesseraud et al., 2011) and even possess secretagogue activities by which amino acids stimulate the release of several pituitary and pancreatic hormones regulating feed intake and growth (Barbul, 1986; Dorshkind and
Table 4. Effects of genotype and L-arginine supply on heart and digestive organs growth from hatch to week 18

|        | WLA       | BLA       | R11       |
|--------|-----------|-----------|-----------|
|        | LA AA HA  | LA AA HA  | LA AA HA  |
| Heart, % |           |           |           |
| hatch   | 0.84A,b   | 0.85A,b   | 0.75A,b   |
| week 6  | 0.62A,b   | 0.65A,b   | 0.65AB,b  |
| week 12 | 0.55A,B,c | 0.64A,B,c | 0.60A,B,c |
| week 18 | 0.51B,ab  | 0.53C,ab  | 0.48C,ab  |
| Liver, %  |           |           |           |
| hatch   | 4.19A,ab  | 3.99A,b   | 3.76A,bc  |
| week 6  | 3.74A,a   | 3.42A,b   | 3.56A,ab  |
| week 12 | 3.26A,c   | 2.90A,b   | 3.08A,bc  |
| week 18 | 2.62A,a   | 2.65A,a   | 2.50A,b   |
| Pancreas, % |           |           |           |
| hatch   | 0.16C,ab  | 0.21C,a   | 0.16B,ab  |
| week 6  | 0.42A,a   | 0.40A,ab  | 0.38A,ab  |
| week 12 | 0.29B,ab  | 0.30B,ab  | 0.23B,b   |
| week 18 | 0.21BC,a  | 0.21C     | 0.23B     |
| Gizzard, % |           |           |           |
| hatch   | 5.56A,a   | 5.35A,b   | 5.32A,b   |
| week 6  | 5.09A,a   | 4.94A,b   | 3.74A,c   |
| week 12 | 3.41B,b   | 3.28B,b   | 3.02C,b   |
| week 18 | 2.56C,b   | 2.52C,b   | 2.93C,b   |

Table 4. Effects of genotype and L-arginine supply on heart and digestive organs growth from hatch to week 18 (Continued)

|        | L68       | ANOVA (p values) |        |        |        |        |        |        |
|--------|-----------|------------------|--------|--------|--------|--------|--------|--------|
|        | LA AA HA  | PESEM            | GT     | DIET   | AGE    | GT × DIET | GT × AGE | DIET × AGE |
| Heart, % |           |                  |        |        |        |        |        |        |
| hatch   | 0.86A,ab  | 0.96A,a          | 0.86A,ab | 0.04    | <0.05  | 0.704   | <0.001  | 0.0599  |
| week 6  | 0.73A,ab  | 0.66B,b          | 0.82A,a | 0.04    | <0.05  | 0.704   | <0.001  | 0.0599  |
| week 12 | 0.63A,b   | 0.60B,b          | 0.54B,b  | 0.04    | <0.05  | 0.704   | <0.001  | 0.0599  |
| week 18 | 0.43C,b   | 0.48C,ab         | 0.52B,ab | 0.04    | <0.05  | 0.704   | <0.001  | 0.0599  |
| Liver, %  |           |                  |        |        |        |        |        |        |
| hatch   | 4.34A,a   | 4.22A,ab         | 4.43A,a | 0.15    | <0.001 | <0.001  | <0.001  | <0.001  |
| week 6  | 3.46A,b   | 3.06B,b          | 3.15B,b  | 0.15    | <0.001 | <0.001  | <0.001  | <0.001  |
| week 12 | 2.88C,b   | 2.29C,c          | 2.46C,c  | 0.15    | <0.001 | <0.001  | <0.001  | <0.001  |
| week 18 | 2.15D,b   | 2.31C,ab         | 2.38C,ab | 0.15    | <0.001 | <0.001  | <0.001  | <0.001  |
| Pancreas, % |           |                  |        |        |        |        |        |        |
| hatch   | 0.16B,ab  | 0.22B,a          | 0.17B,ab | 0.02    | <0.05  | 0.076   | <0.001  | <0.011  |
| week 6  | 0.43A,a   | 0.38A,ab         | 0.42A,a  | 0.02    | <0.05  | 0.076   | <0.001  | <0.011  |
| week 12 | 0.22B,b   | 0.25B,ab         | 0.24B,ab | 0.02    | <0.05  | 0.076   | <0.001  | <0.011  |
| week 18 | 0.23B     | 0.23B            | 0.19B    | 0.02    | <0.05  | 0.076   | <0.001  | <0.011  |
| Gizzard, % |           |                  |        |        |        |        |        |        |
| hatch   | 4.77A,b   | 4.94A,b          | 5.18A,b  | 0.30    | <0.001 | <0.001  | <0.001  | <0.001  |
| week 6  | 4.61A,b   | 4.33A,b          | 4.43B,b  | 0.30    | <0.001 | <0.001  | <0.001  | <0.001  |
| week 12 | 3.47B,ab  | 3.37B,b          | 3.89B,C,a | 0.30    | <0.001 | <0.001  | <0.001  | <0.001  |
| week 18 | 3.17B,ab  | 3.30B,a          | 3.23C,a  | 0.30    | <0.001 | <0.001  | <0.001  | <0.001  |

WLA: high performing White Leghorn; BLA: high performing Rhode Island Red; R11: low performing White Leghorn; L68: low performing New Hampshire; LA, AA, HA: low, adequate and high L-arginine supplied diets; PSEM: pooled standard error of means; GT: genotype

A-D: LSMeans values with PSEM (n=3 chicks/group) of one organ in the same column without common superscripts differ significantly (p<0.05)

a-c: LSMeans values with PSEM (n=3 chicks/group) in the same row without common superscripts differ significantly (p<0.05)
Therefore, this study aimed to give insight into organs’ growth response and sensitivity to a graded dietary Arg supply in a distinct chicken model from hatch to 18 weeks of age.

As the availability of plasma Arg depends on chicken’s dietary intake of Arg directly (Chu and Nesheim, 1979; Kwak et al., 1999, 2001), Arg-involved mechanisms regulating feed intake and subsequent growth are immediately affected by dietary Arg (Kidd et al., 2001; Jahanian, 2009; Wang et al., 2014a, 2014b; Lieboldt et al., 2016).

In the present study, deficient dietary Arg tended to induce feed intake depression, whereas surplus dietary Arg even stimulated feed intake in reared chicken genotypes. The feed

| Table 5. Effects of genotype and L-arginine supply on lymphoid organ growth from hatch to week 18 (Continued) |
|---------------------------------|---------|---------|
| L68                             | ANOVA (p values) |
| LA | AA | HA | PSEM | GT | DIET | AGE | GT×DIET | GT×AGE | DIET×AGE |
| Bursa, %                        |          |
| hatch                           |          |
| week 6                          | 0.09C,b  | 0.13C,ab | 0.06C,b | <0.001 | 0.024 | <0.001 | <0.001 | 0.002 |
| week 12                         | 0.29A,b  | 0.28B,c  | 0.28A,b  | <0.001 | 0.073 | <0.001 | <0.001 | 0.005 |
| week 18                         | 0.28A,b  | 0.28B,c  | 0.28A,b  | <0.001 | 0.010 | <0.001 | <0.001 | 0.024 |

| Thymus, %                       |          |
| hatch                           |          |
| week 6                          | 0.30B,c  | 0.29B,c  | 0.28B,c  | <0.001 | 0.014 | <0.001 | <0.001 | 0.024 |
| week 12                         | 0.30B,c  | 0.29B,c  | 0.28B,c  | <0.001 | 0.014 | <0.001 | <0.001 | 0.024 |
| week 18                         | 0.28B,c  | 0.28B,c  | 0.28B,c  | <0.001 | 0.014 | <0.001 | <0.001 | 0.024 |

| Spleen, %                       |          |
| hatch                           |          |
| week 6                          | 0.29B,c  | 0.29B,c  | 0.28B,c  | <0.001 | 0.019 | <0.001 | <0.001 | 0.024 |
| week 12                         | 0.29B,c  | 0.29B,c  | 0.28B,c  | <0.001 | 0.019 | <0.001 | <0.001 | 0.024 |
| week 18                         | 0.29B,c  | 0.29B,c  | 0.28B,c  | <0.001 | 0.019 | <0.001 | <0.001 | 0.024 |

WLA: high performing White Leghorn; BLA: high performing Rhode Island Red; R11: low performing White Leghorn; L68: low performing New Hampshire; LA, AA, HA: low, adequate and high L-arginine supplied diets; PSEM: pooled standard error of means; GT: genotype
A-C: LSMeans values with PSEM (n=3 chicks/group) of one organ in the same column without common superscripts differ significantly (p<0.05)
a-d: LSMeans values with PSEM (n=3 chicks/group) in the same row without common superscripts differ significantly (p<0.05)
intake regulating properties of dietary Arg derive from two Arg-depending pathways mainly: Firstly, NO serves as appetitie regulating neuronal mediator whose concentration depends directly on available plasma Arg, and dietary Arg in turn (Choi et al., 1994, 1997; Khan et al., 2007; Wang et al., 2014a). The authors have further described that surplus dietary Arg elevates NO levels stimulating appetite and feed intake subsequently. On the contrary, insufficient dietary Arg lowers NO levels and alters hypothalamic protein expression inducing appetite inhibition further (Basoo et al., 2012; Wang et al., 2014a, 2014b). Secondly, Arg possesses secretagogue activities that stimulate the release of growth and feed intake regulating pancreatic and putitary hormones including glucagon, insulin, insulin-growth-factor-1 (IGF-1), somatotropin and neuropeptides among others (Barbul, 1986; Gaskin et al., 2003; Farr et al., 2005; Yang et al., 2007; Scanes, 2009). Depending on the type of released hormone Arg can alter carbohydrate, protein and lipid metabolism as well as feed consumption and body growth secondary (Rocha et al., 1972; Palmer et al., 1975; Meijer and Dubbelhuis, 2004).

Due to Arg-induced alterations in feed intake, body growth of deficiently Arg fed chickens decreased secondary, whereas growth of high performing genotypes even increased in oversupplied Arg fed chickens. On the contrary, low performing R11 did not respond to surplus dietary Arg, but L68 even showed growth depression. These differences lead to the assumption that genotypes possess varying sensitivities to dietary Arg and differ in their Arg requirements for optimal growth subsequently (Nesheim and Hutt, 1962; Hutt and Nesheim, 1966; Kwak et al., 2001; Lieboldt et al., 2016). The growth-regulating properties of Arg refer to its function as primary component of body protein and creatine, as precursor of connective tissue forming L-proline and hydroxy-proline (Popovic et al., 2007) and as precursor of growth-promoting polyamines encouraging cell proliferation by enhanced DNA, RNA and protein synthesis as well as uptake of amino acids into cells (Pegg and McCann, 1982; Smith, 1990). Additionally, the sensitive dietary and metabolic interactions between Arg and lysine as well as Arg and methionine can act as growth-limiting factors in chickens (D’Mello and Lewis, 1970; Keshavarz and Fuller, 1971; Austic and Calvert, 1981).

Depending on their genetic background and age (Lieboldt et al., 2015, 2016) studied chicken lines differed between growth parameters markedly. In poultry research age-dependent body growth is usually evaluated using the Gompertz equation (Gous et al., 1999; Sakomura et al., 2005; Lieboldt et al., 2015, 2016), whereas that of organs and tissues is frequently calculated using the allometric growth function (Huxley and Teissier, 1936; Ono et al., 1993; Govaerts et al., 2000; Zelenka et al., 2011). The allometric growth coefficient b gives valuably biological information on organ development in relation to that of whole body weight and allows the classification of organs in earlier (b<1), equal to (b=1) or later maturing (b>1) than whole body weight (Larbier and Leclercq, 1994). Based on b<1 and the age-related decline in their relative weights, the heart, liver and gizzard as well as the bursa and pancreas except for the pancreas of HA fed BLA could be considered as early maturing organs. However, the heart, liver and bursa reached their maturity later than the gizzard and pancreas. Gouvaerts et al. (2000) have associated this precocious development of gizzard and pancreas with their primary digestive function and their subsequent importance in supplying the avian organism with energy and nutrients for growth. Although differences in allometric growth coefficients can be found between the present study and Gouvaerts et al. (2000), the direction of b has been the same and differences refer to genetic, nutritional and age-related variations between the studies.

Moreover, the spleen and thymus also belonged to the early maturing organs (b<1) except for BLA in general and the spleen of WLA and R11 as well as the thymus of R11 and L68 when fed with insufficient dietary Arg. The allometric growth coefficient of these organs took values of b>1 and indicated a growth-retarding effect of deficient dietary Arg on body weight and organ weights. As bursa growth did not retard in Arg insufficiently fed chicks, it can be concluded that lymphoid organs respond differently to dietary Arg limitations and that the bursa is less sensitive to deficient Arg than thymus and spleen.

In accordance to Plavnik and Hurwitz (1982) and Gouvaerts et al. (2000), relative weights of heart, liver and gizzard decreased continuously. Based on their equally directed growth behaviour expressed by a strong positive correlation between each other, these organs could be summarized to a single group. On the contrary, the pancreas and the lymphoid organs spleen, thymus and bursa formed another group. Although organ growth was equally directed within each organ group, the sensitivity to dietary Arg differed between both organ groups as well as within them. This leads to the assumption that each internal organ has its own specific sensitivity to dietary Arg that might be mediated through the organ-specific expression of Arg up taking membrane transporters, the cationic amino acid transporters (CAT) as described by Humphrey et al. (2004) and Humphrey and Klasing (2005). In the second organ group lymphoid organs and pancreas increased in their relative weights after hatch, peaked from week 6 to 12 and decreased until week 18. After achieving their maximum size from week 8 to 12 thymus and bursa involute physiologically and disappear largely by sexual maturity (Ciriacolo et al., 2003). Because lymphoid organs are very sensitive to different kinds of stress (Puvadolpirod and Thaxton, 2000) the thymus size serves as sensitive indicator of health and stress response (Shelat et al., 1997). Although Kwak et al. (1999), Kidd et al. (2001) and the present study have not shown further thymus and bursa weight promoting effects beyond recommended Arg supply, Barbul et al. (1981a, 1981b) and Daly et al. (1990) have described beneficial effects of dietary Arg supplementations in mammals with increasing thymus weight and cellularity. Dorslikind and Horsemman (2000) and Calder and Yaqoob
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