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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.

Key words: 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

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and Ratner, 1963). Therefore, chickens are highly depended on dietary Arg influencing the availability of plasma Arg directly (Chu and Nesheim, 1979; Kwak *et al*., 1999, 2001). Because of its function as precursor of proteins, creatine, polyamines, L-proline and nitric oxides (NO; reviewed in: Khajali and Wideman, 2010) Arg plays a pivotal role in multiple processes such as growth (Kidd *et al*., 2001; Lieboldt *et al*., 2016) and immune response (Sung *et al*., 1991; Kwak *et al*., 2001; Deng *et al*., 2005; Jahanian, 2009). The production of NO through different isoforms of nitric oxide synthase (NOS) is substrate-limited by Arg (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 dietary 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.

Chickens 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 chickens' 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-

Table 1. **Ingredients, analysed and calculated chemical composition of the experimental diets**

Ingredients (g/kg diet)	Chicks and growers (week 1–7)			Growers and pullets (week 8–18)		
	LA	AA	HA	LA	AA	HA
Barley	200.0	200.0	200.0	300.0	300.0	300.0
Wheat	100.0	100.0	100.0	150.0	150.0	150.0
Triticale	—	—	—	147.5	147.5	147.5
Corn	399.0	396.0	386.0	209.5	208.5	201.5
Corn gluten meal	150.0	150.0	150.0	80.0	80.0	80.0
Lucerne pellets	50.0	50.0	50.0	60.0	60.0	60.0
Wheat bran	39.8	39.8	39.8	—	—	—
Soybean oil	10.0	10.0	10.0	10.0	10.0	10.0
Calcium carbonate	—	—	—	7.0	7.0	7.0
Calcium phosphate	33.3	33.3	33.3	20.0	20.0	20.0
Premix[1]	10.0	10.0	10.0	—	—	—
Premix[2]	—	—	—	10.0	10.0	10.0
L-lysine HCl	4.9	4.9	4.9	2.6	2.6	2.6
L-arginine	—	3.0	13.0	—	1.0	8.0
Sodium chloride	3.0	3.0	3.0	3.4	3.4	3.4
chemical composition, g/kg diet						
Dry matter[3]	897.6	893.0	896.6	888.4	891.4	891.7
Crude ash[3]	60.0	57.2	58.2	52.1	53.2	54.2
Crude protein[4]	174.1	176.0	186.9	128.1	132.5	136.2
Kjeldahl Nitrogen[3]	27.9	28.5	32.0	20.5	21.9	23.1
Crude fat[3]	40.1	38.6	37.2	30.7	31.6	34.6
Crude fibre[3]	33.6	31.0	34.2	38.5	39.3	39.3
Starch[3]	459.7	457.7	449.5	493.2	491.8	482.8
Sucrose[3]	20.6	20.1	20.3	23.8	24.3	23.0
Phosphorous[3]	9.8	10.0	10.2	13.9	14.3	13.8
Calcium[3]	12.7	12.6	13.1	11.5	11.8	11.6
AME_N (MJ/kg)[5]	12.0	12.0	12.0	11.6	11.6	11.6
Methionine[6]	3.3	3.3	3.3	2.4	2.4	2.4
Lysine[6]	9.0	9.0	8.9	6.3	6.3	6.2
Arginine[6]	6.5	9.5	19.5	4.7	6.5	13.4

[1] Premix – chicks: feed additives (per kg premix): Vitamin A, 1,200,000 IU; Vitamin D_3, 350,000 IU; Vitamin E, 4,000 mg; Vitamin B_1, 250 mg; Vitamin B_2, 800 mg; Vitamin B_6, 600 mg; Vitamin B_{12}, 3,200 μg; Vitamin K_3, 450 mg; Nicotin amide, 4,500 mg; Calcium-D-pantothenate, 1,500 mg; Folic acid, 120 mg; Biotin, 5,000 μg; Choline chloride, 55,000 mg; Fe, 3,200 mg; Cu, 1,200 mg; Mn, 10,000 mg; Zn, 8,000 mg; I, 160 mg; Se, 40 mg; Co, 20 mg; Butylated hydroxy toluene (BHT), 10,000 mg

[2] Premix – pullets: feed additives (per kg premix): Vitamin A, 1,000,000 IU; Vitamin D_3, 200,000 IU; Vitamin E, 2,500 mg; Vitamin B_1, 250 mg; Vitamin B_2, 500 mg; Vitamin B_6, 400 mg; Vitamin B_{12}, 1,850 μg; Vitamin K_3, 300 mg; Nicotin amide, 3,000 mg; Calcium-D-pantothenate, 900 mg; Folic acid, 80 mg; Biotin, 2,100 μg; Choline chloride, 30,000 mg; Fe, 4,000 mg; Cu, 1,500 mg; Mn, 8,000 mg; Zn, 8,000 mg; I, 160 mg; Se, 32 mg; Co, 20 mg; Butylated hydroxy toluene (BHT), 10,000 mg

[3] Analysed

[4] Calculation based on the analysed Kjeldahl nitrogen (N). Crude protein of basal diets (LA) was calculated by multiplying Kjeldahl N by 6.25. As N content of free Arg is twice as high as that of crude protein the N difference between Arg supplemented diets (AA and HA) and basal diet (LA) was multiplied by 3.13 and added to that of the basal diet

[5] Apparent metabolisable energy concentrations corrected to zero nitrogen balance (AME_N), calculated according to the energy estimation equation of the WPSA (Vogt, 1986)

[6] Calculated based on analysed amino acid contents of ingredients and their proportions of the diets

centration corrected to zero N balance (AME_N) 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 Teissler (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 (Larbier 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 covariance 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 separately for each time using the "pdiff" statement. The results were reported as least square means with their pooled standard errors of the means (PSEM).

Results

Growth Parameters

In Table 2 the growth-related traits of reared chickens are presented in six-week-intervals from hatch to 18 weeks of age. At hatch BW did not differ between experimental groups. In the following, BW and DWG increased age-dependently ($p_{age}<0.001$) and L68 achieved higher BW and DWG than the other genotypes from week 6 onwards (BW: $p_{genotype}<0.001$; DWG: $p_{genotype}<0.05$). Because DWG of BLA did not change during the entire trial, white genotypes gained higher BW than BLA from week 6 to 12 ($p_{genotype*age}<0.001$). In all genotypes except for BLA, DWG declined from week 12 onwards and white genotypes differed from each other at week 18 only ($p<0.001$). Although a dietary effect on DWG was not found ($p_{diet}=0.625$), LA fed chicks reached lower absolute BW than those fed with AA and HA ($p_{diet}<0.05$). Latter one tended to cause higher BW than LA generally ($p=0.068$) and induced a higher BW than LA and AA in high performing pullets at week 18 ($p<0.01$). On the contrary, R11 did not differ in BW diet-dependently and HA fed L68 chicks weighed less than LA and AA fed L68 chicks ($p<0.05$).

Moreover, WLA and L68 consumed more feed than R11 and BLA ($p_{genotype}<0.001$). Although DFI increased age-dependently ($p_{age}<0.001$; $p_{genotype*age}<0.001$), L68 took up most feed and R11 showed the lowest DFI within genotypes during the entire trial. In addition, high performing genotypes differed from R11 from week 6 to 18 ($p<0.001$). HA caused higher DFI than LA and AA ($p_{diet}<0.01$), whereas LA even tended to induce lower DFI than AA from week 6 onwards ($p_{diet*age}=0.077$).

Besides, the FCR was only affected by "age" ($p_{age}<0.01$). While the first and second six-week-interval did not differ between each other, the FCR increased significantly from week 12 to 18 ($p<0.01$).

Allometric Organ Growth

Table 3 presents the parameters of organ-specific allometric growth functions fitted regressively to absolute BW recorded from hatch to 18 weeks of age. In order to illustrate differences between experimental groups graphically, Figure 1 shows the calculated allometric growth curves of the bursa *cloacalis* 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**

	WLA			BLA			R11		
	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	$762^{B, d}$	$855^{B, c}$	$930^{B, b}$	$645^{B, e}$	$691^{B, e}$	$656^{B, e}$	$784^{B, d}$	$872^{B, bc}$	$834^{B, cd}$
week 18	$957^{A, e}$	$1034^{A, d}$	$1104^{A, c}$	$895^{A, f}$	$941^{A, ef}$	$1027^{A, d}$	$953^{A, e}$	$938^{A, ef}$	$954^{A, e}$
Daily weight gain, g/chick/d									
hatch to week 6	$6.2^{B, ab}$	$7.9^{B, ab}$	$7.3^{B, ab}$	6.3^{ab}	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	$49.7^{B, c}$	$53.8^{B, b}$	$52.1^{B, bc}$	$49.6^{B, c}$	$52.0^{B, bc}$	$51.9^{B, bc}$	$46.1^{B, d}$	$46.9^{B,cd}$	$46.0^{B, d}$
week 12 to 18	$68.5^{A, c}$	$70.0^{A, c}$	$69.6^{A, c}$	$65.4^{A, d}$	$65.4^{A, d}$	$67.3^{A, cd}$	$61.9^{A, e}$	$63.7^{A,de}$	$63.4^{A, de}$
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	3.98^{B}	3.31^{B}	4.39^{B}
week 6 to 12	4.44^{B}	4.64^{B}	3.75^{B}	6.05^{AB}	6.19^{AB}	7.41	3.75^{B}	3.45^{B}	3.31^{B}
week 12 to 18	14.89^{A}	16.28^{A}	16.98^{A}	10.90^{A}	10.90^{A}	7.65	15.48^{A}	14.81^{A}	16.26^{A}

Table 2. **Effects of genotype and L-arginine supply on growth parameters from hatch to week 18 (Continued)**

	L68			PSEM	ANOVA (p values)					
	LA	AA	HA		GT	DIET	AGE	GT×DIET	GT×AGE	DIET×AGE
Body weight, g/chick										
hatch	40^{D}	36^{D}	39^{D}							
week 6	$321^{C, bc}$	$417^{C, a}$	$336^{C, b}$	30	<0.001	<0.05	<0.001	0.121	<0.001	0.453
week 12	$898^{B, bc}$	$1060^{B, a}$	$848^{B, cd}$							
week 18	$1248^{A, ab}$	$1262^{A, a}$	$1172^{A, b}$							
Daily weight gain, g/chick/d										
hatch to week 6	$6.7^{B, ab}$	$9.1^{B, a}$	$7.1^{B, ab}$	1.5	<0.05	0.625	<0.001	0.928	<0.001	0.724
week 6 to 12	$13.7^{A, ab}$	$15.3^{A, a}$	$12.2^{A, b}$							
week 12 to 18	$8.3^{B, a}$	$4.8^{C, b}$	$7.7^{B, a}$							
Daily feed intake, g/chick/d										
hatch to week 6	$24.2^{C, a}$	$23.7^{C, a}$	$25.3^{C, a}$	1.3	<0.001	<0.01	<0.001	0.364	<0.001	*0.077*
week 6 to 12	$54.3^{B, b}$	$61.3^{B, a}$	$60.1^{B, a}$							
week 12 to 18	$75.9^{A, b}$	$79.8^{A, a}$	$76.7^{A, ab}$							
Feed conversion ratio, g/g										
hatch to week 6	3.61	2.60^{B}	3.56	2.4	0.385	0.315	<0.01	0.166	0.371	0.299
week 6 to 12	3.96	4.01^{B}	4.93							
week 12 to 18	9.14	16.63^{A}	9.96							

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 ($p<0.05$)

Table 3. **Results of nonlinear regression of the allometric growth function[1] fitted to body weight dependent organ weights from hatch to week 18**

		Heart				Liver				Pancreas				Gizzard			
		a	b	R^2	RSD	a	b	R^2	RSD	a	b	R^2	RSD	a	b	R^2	RSD
WLA	LA	0.015	0.844	0.990	0.45	0.193	0.717	0.966	4.50	0.041	0.584	0.907	0.64	0.599	0.554	0.938	5.93
	AA	0.021	0.809	0.920	1.57	0.112	0.796	0.976	4.04	0.037	0.609	0.877	0.84	0.655	0.544	0.913	7.58
	HA	0.019	0.822	0.960	1.13	0.188	0.721	0.965	5.22	0.032	0.621	0.930	0.61	0.164	0.752	0.985	3.57
BLA	LA	0.019	0.828	0.987	0.52	0.201	0.692	0.973	3.20	0.018	0.705	0.948	0.46	0.215	0.711	0.967	4.34
	AA	0.033	0.722	0.952	0.90	0.079	0.833	0.973	3.45	0.012	0.789	0.981	0.32	0.206	0.731	0.974	4.27
	HA	0.013	0.877	0.985	0.60	0.115	0.776	0.986	2.57	0.001	1.203	0.974	0.34	0.079	0.878	0.971	5.10
R11	LA	0.034	0.728	0.964	0.89	0.174	0.719	0.956	4.76	0.010	0.812	0.957	0.53	0.302	0.682	0.959	6.17
	AA	0.041	0.685	0.965	0.81	0.149	0.735	0.956	4.67	0.031	0.617	0.900	0.65	0.361	0.631	0.920	7.46
	HA	0.039	0.702	0.918	1.36	0.133	0.754	0.960	4.64	0.018	0.700	0.925	0.62	0.267	0.673	0.969	4.57
L68	LA	0.063	0.638	0.905	1.72	0.248	0.667	0.962	5.12	0.020	0.693	0.915	0.77	0.238	0.716	0.981	4.85
	AA	0.013	0.877	0.964	1.24	0.051	0.891	0.966	5.21	0.025	0.672	0.957	0.58	0.172	0.768	0.982	5.21
	HA	0.039	0.712	0.966	0.99	0.103	0.793	0.987	2.85	0.044	0.565	0.801	1.03	0.221	0.733	0.951	7.95

Table 3. **Results of nonlinear regression of the allometric growth function[1] fitted to body weight dependent organ weights from hatch to week 18 (Continued)**

		Spleen				Thymus				Bursa			
		a (x 10^{-1})	b	R^2	RSD	a (x 10^{-2})	b	R^2	RSD	a	b	R^2	RSD
WLA	LA	0.018	1.017	0.981	0.25	1.179	0.882	0.926	1.35	0.013	0.842	0.894	1.34
	AA	0.071	0.801	0.981	0.24	1.740	0.840	0.945	1.33	0.022	0.751	0.901	1.26
	HA	0.031	0.927	0.978	0.30	1.344	0.878	0.911	1.90	0.022	0.751	0.846	1.68
BLA	LA	0.006	1.194	0.997	0.11	0.048	1.292	0.903	0.95	0.009	0.813	0.878	0.80
	AA	0.014	1.071	0.950	0.44	0.006	1.586	0.832	1.29	0.056	0.521	0.787	0.87
	HA	0.006	1.203	0.974	0.34	0.015	1.464	0.885	1.20	0.024	0.646	0.864	0.73
R11	LA	0.005	1.193	0.635	1.13	0.215	1.041	0.798	1.19	0.007	0.872	0.919	0.76
	AA	0.007	1.124	0.707	1.09	0.311	0.980	0.829	1.20	0.011	0.773	0.837	1.84
	HA	0.047	0.833	0.668	1.04	0.720	0.856	0.759	1.47	0.003	0.993	0.901	0.81
L68	LA	0.067	0.875	0.941	0.81	0.003	1.749	0.946	1.45	0.009	0.856	0.932	1.01
	AA	0.051	0.913	0.966	0.63	5.375	0.600	0.875	1.36	0.011	0.800	0.872	1.24
	HA	0.038	0.933	0.943	0.63	0.834	0.888	0.971	0.70	0.011	0.816	0.853	1.37

[1] $y(BW) = a * BW^b$ with y(BW)=organ weight in g at body weight (BW) in g; a, b: regression coefficients; b: allometric growth coefficient; 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; R^2=coefficient of determination; RSD=residual standard deviation

($p_{genotype} < 0.001$; $p_{genotype*age} < 0.001$), 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 ($p_{diet} = 0.704$), LA caused higher liver proportions than AA and HA ($p_{diet} < 0.01$).

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 ($p_{age} < 0.001$; $p_{genotype*age} < 0.001$; $p_{diet*age} < 0.05$). 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

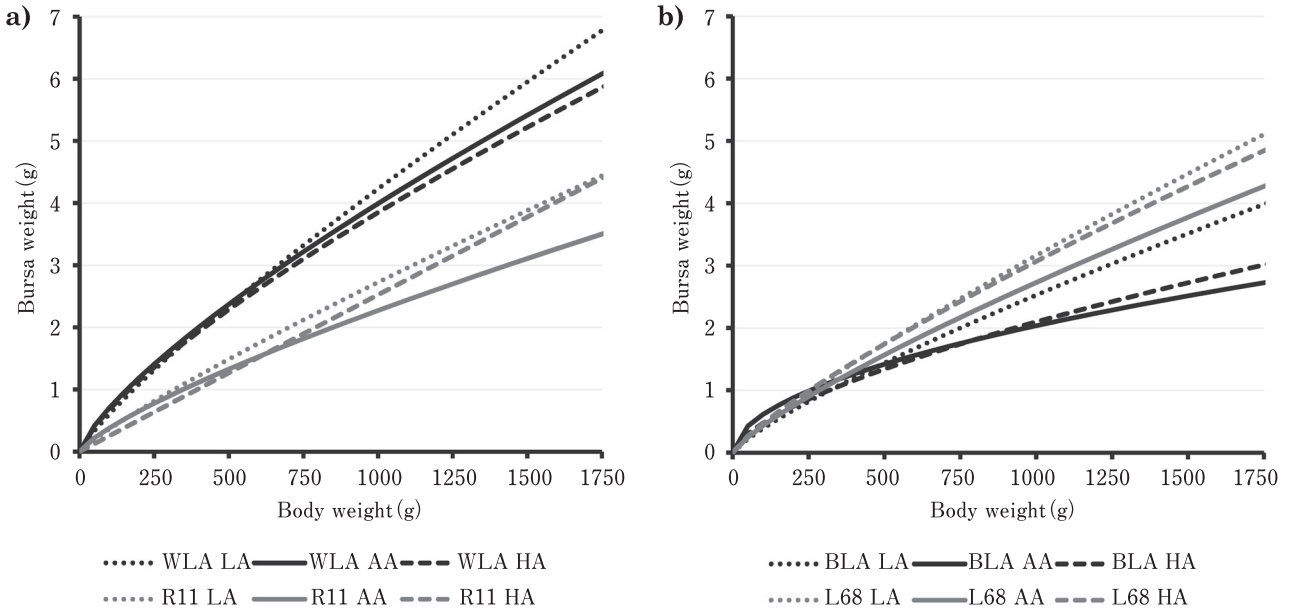

Fig. 1. **Graphic presentation of calculated allometric organ growth exemplary for the bursa *cloacalis* in high (WLA) and low (R11) performing white genotypes (a) and in high (BLA) and low (L68) performing brown genotypes (b) of purebred layer lines supplied with graded dietary L-arginine.** Note: LA, AA, HA: low, adequate and high L-arginine supplied diets.

were larger than those of R11 and BLA ($p_{genotype}<0.001$; $p_{genotype*age}<0.001$). 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 ($p_{age}<0.001$; $p_{genotype*age}<0.001$). 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 ($p_{diet}=0.052$). On the contrary, AA and HA tended to induce higher relative thymus weights in WLA than LA ($p_{genotype*diet}=0.061$). 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 ($p_{diet*age}<0.05$).

Finally, highest and lowest spleen proportions were found in low performing chickens ($p_{genotype}<0.001$; $p_{genotype*diet}<0.05$). 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 ($p_{age}<0.001$; $p_{genotype*age}<0.001$). 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 ($p_{diet}<0.01$).

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	$0.84^{A,b}$	$0.85^{A,b}$	$0.75^{A,b}$	$0.78^{A,b}$	$0.75^{A,b}$	$0.76^{A,b}$	$0.97^{A,a}$	$0.87^{A,ab}$	$0.96^{A,a}$
week 6	$0.62^{B,b}$	$0.65^{B,b}$	$0.65^{AB,b}$	$0.70^{AB,b}$	$0.67^{AB,b}$	$0.62^{B,b}$	$0.69^{B,b}$	$0.70^{B,b}$	$0.70^{B,b}$
week 12	$0.55^{B,ab}$	$0.64^{BC,a}$	$0.60^{B,ab}$	$0.64^{B,a}$	$0.58^{B,ab}$	$0.60^{B,ab}$	$0.59^{BC,ab}$	$0.52^{C,b}$	$0.62^{B,ab}$
week 18	$0.51^{B,ab}$	$0.53^{C,ab}$	$0.48^{C,ab}$	$0.56^{B,a}$	$0.46^{C,ab}$	$0.53^{B,ab}$	$0.51^{C,ab}$	$0.45^{C,ab}$	$0.44^{C,b}$
Liver, %									
hatch	$4.19^{A,ab}$	$3.99^{A,b}$	$3.76^{A,bc}$	$3.74^{A,bc}$	$3.83^{A,bc}$	$3.48^{A,c}$	$4.10^{A,ab}$	$4.10^{A,ab}$	$3.79^{A,bc}$
week 6	$3.74^{B,a}$	$3.42^{B,ab}$	$3.56^{A,ab}$	$3.46^{A,ab}$	$3.05^{B,b}$	$3.05^{B,b}$	$3.45^{B,ab}$	$3.23^{B,b}$	$3.20^{B,b}$
week 12	$3.26^{C,a}$	$2.90^{C,ab}$	$3.08^{B,ab}$	$2.99^{B,ab}$	$2.70^{BC,bc}$	$2.79^{BC,bc}$	$3.03^{C,ab}$	$2.78^{C,bc}$	$2.87^{B,b}$
week 18	$2.62^{D,a}$	$2.65^{C,a}$	$2.50^{C,ab}$	$2.34^{C,ab}$	$2.46^{C,ab}$	$2.43^{C,ab}$	$2.30^{D,ab}$	$2.20^{D,b}$	$2.24^{C,b}$
Pancreas, %									
hatch	$0.16^{C,ab}$	$0.21^{C,a}$	$0.16^{B,ab}$	$0.17^{B,ab}$	$0.18^{C,ab}$	$0.17^{B,ab}$	$0.09^{B,b}$	$0.13^{C,b}$	$0.12^{C,b}$
week 6	$0.42^{A,a}$	$0.40^{A,ab}$	$0.38^{A,ab}$	$0.34^{A,ab}$	$0.35^{A,ab}$	$0.33^{A,b}$	$0.33^{A,b}$	$0.40^{A,ab}$	$0.36^{A,ab}$
week 12	$0.29^{B,ab}$	$0.30^{B,ab}$	$0.23^{B,b}$	$0.32^{A,a}$	$0.30^{AB,ab}$	$0.32^{A,a}$	$0.31^{A,a}$	$0.24^{B,ab}$	$0.27^{B,ab}$
week 18	0.21^{BC}	0.21^{C}	0.23^{B}	0.23^{B}	0.27^{B}	0.23^{B}	0.26^{A}	0.20^{BC}	0.22^{B}
Gizzard, %									
hatch	$5.56^{A,a}$	$5.35^{A,ab}$	$5.32^{A,ab}$	$5.33^{A,ab}$	$5.76^{A,a}$	$5.31^{A,ab}$	$4.67^{AB,bc}$	$4.25^{A,c}$	$4.40^{A,bc}$
week 6	$5.09^{A,a}$	$4.94^{A,ab}$	$3.74^{B,c}$	$4.19^{B,bc}$	$4.79^{B,ab}$	$3.74^{B,c}$	$4.99^{A,ab}$	$4.59^{A,ab}$	$4.61^{A,ab}$
week 12	$3.41^{B,b}$	$3.28^{B,b}$	$3.02^{C,b}$	$3.34^{C,b}$	$3.20^{C,b}$	$3.61^{B,ab}$	$4.06^{B,a}$	$3.45^{B,b}$	$3.17^{B,b}$
week 18	$2.56^{C,b}$	$2.52^{C,b}$	$2.93^{C,ab}$	$3.12^{C,ab}$	$3.39^{C,a}$	$3.36^{B,a}$	$3.20^{C,a}$	$2.54^{C,b}$	$2.69^{B,b}$

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	PSEM	GT	DIET	AGE	GT×DIET	GT×AGE	DIET×AGE
Heart, %										
hatch	$0.86^{A,ab}$	$0.96^{A,a}$	$0.86^{A,ab}$							
week 6	$0.73^{A,ab}$	$0.66^{B,b}$	$0.82^{A,a}$	0.04	<0.05	0.704	<0.001	<0.05	<0.001	0.599
week 12	$0.63^{B,ab}$	$0.60^{B,ab}$	$0.54^{B,ab}$							
week 18	$0.43^{C,b}$	$0.48^{C,ab}$	$0.52^{B,ab}$							
Liver, %										
hatch	$4.34^{A,a}$	$4.22^{A,ab}$	$4.43^{A,a}$							
week 6	$3.46^{B,ab}$	$3.06^{B,b}$	$3.15^{B,b}$	0.15	<0.001	<0.01	<0.001	0.502	<0.001	<0.01
week 12	$2.88^{C,b}$	$2.29^{C,c}$	$2.46^{C,c}$							
week 18	$2.15^{D,b}$	$2.31^{C,ab}$	$2.38^{C,ab}$							
Pancreas, %										
hatch	$0.16^{B,ab}$	$0.22^{B,a}$	$0.17^{B,ab}$							
week 6	$0.43^{A,a}$	$0.38^{A,ab}$	$0.42^{A,a}$	0.02	<0.05	*0.076*	<0.001	0.711	<0.001	<0.05
week 12	$0.22^{B,b}$	$0.25^{B,ab}$	$0.24^{B,ab}$							
week 18	0.23^{B}	0.23^{B}	0.19^{B}							
Gizzard, %										
hatch	$4.77^{A,bc}$	$4.94^{A,b}$	$5.18^{A,ab}$							
week 6	$4.61^{A,ab}$	$4.33^{A,bc}$	$4.43^{B,b}$	0.30	0.170	0.320	<0.001	<0.01	<0.001	<0.05
week 12	$3.47^{B,ab}$	$3.37^{B,b}$	$3.89^{BC,a}$							
week 18	$3.17^{B,ab}$	$3.30^{B,a}$	$3.23^{C,a}$							

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 ($p<0.05$)

Table 5. **Effects of genotype and L-arginine supply on lymphoid organ growth from hatch to week 18**

	WLA			BLA			R11		
	LA	AA	HA	LA	AA	HA	LA	AA	HA
Bursa, %									
hatch	$0.19^{C,a}$	$0.15^{C,ab}$	$0.12^{C,ab}$	$0.09^{B,b}$	$0.13^{C,ab}$	$0.06^{C,b}$	$0.08^{B,b}$	$0.06^{C,b}$	$0.13^{B,ab}$
week 6	$0.48^{A,a}$	$0.51^{A,a}$	$0.50^{A,a}$	$0.28^{A,c}$	$0.39^{A,b}$	$0.31^{A,bc}$	$0.31^{A,bc}$	$0.26^{AB,c}$	$0.27^{A,c}$
week 12	$0.51^{A,a}$	$0.45^{AB,a}$	$0.45^{A,a}$	$0.31^{A,bc}$	$0.28^{B,c}$	$0.28^{AB,c}$	$0.31^{A,bc}$	$0.30^{A,c}$	$0.28^{A,c}$
week 18	$0.38^{B,a}$	$0.37^{B,a}$	$0.32^{B,ab}$	$0.26^{A,bc}$	$0.18^{C,c}$	$0.20^{B,c}$	$0.26^{A,bc}$	$0.19^{B,c}$	$0.23^{A,bc}$
Thymus, %									
hatch	$0.40^{C,ab}$	$0.45^{B,a}$	$0.40^{C,ab}$	$0.29^{B,bc}$	$0.31^{A,bc}$	$0.27^{B,c}$	$0.16^{B,d}$	$0.21^{B,cd}$	$0.26^{B,c}$
week 6	$0.53^{B,b}$	$0.66^{A,a}$	$0.63^{A,a}$	$0.34^{AB,d}$	$0.38^{A,d}$	$0.36^{A,d}$	$0.24^{B,e}$	$0.23^{B,e}$	$0.20^{B,e}$
week 12	$0.63^{A,a}$	$0.65^{A,a}$	$0.67^{A,a}$	$0.25^{B,c}$	$0.21^{B,c}$	$0.21^{B,c}$	$0.37^{A,b}$	$0.35^{A,b}$	$0.38^{A,b}$
week 18	$0.47^{BC,a}$	$0.53^{B,a}$	$0.51^{B,a}$	$0.39^{A,b}$	$0.36^{A,b}$	$0.39^{A,b}$	$0.21^{B,c}$	$0.21^{B,c}$	$0.20^{B,c}$
Spleen, %									
hatch	$0.10^{B,a}$	$0.06^{C,b}$	$0.04^{C,b}$	$0.05^{B,b}$	$0.09^{C,ab}$	$0.05^{B,b}$	$0.04^{C,b}$	$0.03^{C,b}$	$0.05^{C,b}$
week 6	$0.22^{A,bc}$	$0.23^{A,b}$	$0.23^{A,b}$	$0.18^{A,c}$	$0.26^{A,ab}$	$0.22^{A,bc}$	$0.11^{B,d}$	$0.11^{B,d}$	$0.10^{B,d}$
week 12	$0.19^{A,c}$	$0.18^{B,c}$	$0.18^{B,c}$	$0.20^{A,bc}$	$0.20^{B,bc}$	$0.20^{A,bc}$	$0.26^{A,ab}$	$0.24^{A,b}$	$0.23^{A,b}$
week 18	$0.20^{A,bc}$	$0.17^{B,c}$	$0.19^{AB,bc}$	$0.22^{A,b}$	$0.22^{AB,b}$	$0.23^{A,ab}$	$0.10^{B,d}$	$0.10^{B,d}$	$0.10^{B,d}$

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	$0.15^{C,ab}$	$0.15^{B,ab}$	$0.09^{C,b}$							
week 6	$0.31^{AB,bc}$	$0.25^{AB,c}$	$0.28^{B,c}$	0.03	<0.001	*0.052*	<0.001	0.586	<0.001	0.502
week 12	$0.39^{A,b}$	$0.33^{A,bc}$	$0.40^{A,b}$							
week 18	$0.29^{B,b}$	$0.23^{B,bc}$	$0.27^{B,bc}$							
Thymus, %										
hatch	$0.39^{A,ab}$	$0.36^{B,b}$	$0.37^{B,ab}$							
week 6	$0.41^{A,cd}$	$0.49^{A,b}$	$0.47^{A,b}$	0.04	<0.001	0.703	<0.001	*0.061*	<0.001	<0.05
week 12	$0.34^{A,b}$	$0.37^{B,b}$	$0.37^{B,b}$							
week 18	$0.38^{A,b}$	$0.31^{B,b}$	$0.39^{AB,b}$							
Spleen, %										
hatch	$0.07^{B,ab}$	$0.11^{B,a}$	$0.06^{B,ab}$							
week 6	$0.29^{A,a}$	$0.30^{A,a}$	$0.27^{A,ab}$	0.02	<0.001	<0.01	<0.001	<0.05	<0.001	0.224
week 12	$0.30^{A,a}$	$0.29^{A,a}$	$0.24^{A,b}$							
week 18	$0.27^{A,a}$	$0.27^{A,a}$	$0.23^{A,ab}$							

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 ($p<0.05$)

Horseman, 2000; Calder and Yaqoob, 2004; Scanes, 2009). 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

intake regulating properties of dietary Arg derive from two Arg-depending pathways mainly: Firstly, NO serves as appetite 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 pituitary 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 (Ciriaco *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. Dorshkind and Horseman (2000) and Calder and Yaqoob

(2004) have considered the release of somatotropin, IGF-1 and prolactin stimulated by Arg secretagogue activities to be responsible for these thymus promoting effects. In case of the bursa *cloacalis*, Humphrey *et al*. (2004) and Humphrey and Klasing (2005) have found a higher mRNA expression of high-affinity CAT than in the thymus under physiological conditions and an increase of total and high-affinity CAT mRNA in bursa and liver during acute phase response with a parallel decreased expression in thymus. On the basis of their findings the authors have concluded that the thymus and its containing T cells may be more susceptible to cationic amino acid deficiencies than bursal lymphocytes (Humphrey *et al*., 2004). In contrast to Kwak *et al*. (1999), but in accordance to Deng *et al*. (2005), the increased bursa proportion in insufficiently Arg supplied chickens of the present study could be regarded as an evidencing indication for the assumption of Humphrey *et al*. (2004).

In addition to the direct growth response of organs to dietary Arg, this amino acid is known to modulate lipid metabolism by reducing abdominal fat content as well as plasma triglyceride and total cholesterol concentrations (Corzo *et al*., 2003; Wu *et al*., 2011; Fouad *et al*., 2013). Insufficiently Arg supplied chickens may lack this dietary advantage and suffer from hepatic lipid accumulation subsequently (Milner, 1979; Fu *et al*., 2005). As a result hepatic protein and energy metabolism can be disturbed and inhibit appropriate chicken growth secondary (Butler, 1976; Julian, 2004). On the other hand, known from studies on broilers suffering from pulmonary hypertension syndrome insufficient dietary Arg reduces NO formation on the endothelial side, promotes vasoconstriction and causes subsequently higher pulmonary vascular resistance (Wideman *et al*., 1995, 1996; Ruiz-Feria *et al*., 2001; Basoo *et al*., 2012). Due to blood congestion and passive venous hyperaemia in the lungs and liver, oedema and reduced organ functions occur (reviewed in: Wideman, 2001). In order to classify the aetiology of the observed relative hepatic weight gain in detail, histological and biochemical analysis of the hepatic parenchyma would be necessary.

In conclusion, the present study gives several indications on the closely interlinked metabolic, endocrine and immunological processes involved in body and organ growth during rearing of young layer-type chickens. The used chicken model comprising different genetic backgrounds has been helpful to get an initial impression of changes in organ growth being more or less dependent on genetics. Beside these physiological changes the present study also emphasises the essential function of Arg in numerous metabolic pathways associated with chicken feed intake, body growth and organ growth, and reveals different sensitivities of growing internal organs to dietary Arg.

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