University of Veterinary Medicine Hannover

Effects of dietary L-arginine on metabolism and immune response in layer-type chickens of different genetic backgrounds under physiological and pathophysiological conditions

Thesis
Submitted in partial fulfilment of the requirements for the degree
-Doctor of Veterinary Medicine-
Doctor medicinae veterinariae
( Dr. med. vet. )

by
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Hannover 2015
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Day of the oral examination:  
20.11.2015
This thesis is dedicated to my family
for their endless love, support and encouragement.

Borders - I have never seen one.
But I have heard they exist
in the minds of some people.

Thor Heyerdahl
Parts of this thesis have already been published, accepted or submitted for publication in the following journals:

Phylogenic versus selection effects on growth development, egg laying and egg quality in purebred laying hens.
*European Poultry Science (EPS), 2015, Volume 79, DOI: 10.1399/eps.2015.89*

*Journal of Poultry Science (JPS), 2015, Volume 53, DOI: 10.2141/jpsa.0150067*

Effects of Graded Dietary L-arginine Supply on Organ Growth in Four Genetically Diverse Layer Lines during Rearing Period.
*Journal of Poultry Science (JPS), in press*

Haematological and febrile response to *Escherichia coli* endotoxin in 12-week-old cockerels of genetically diverse layer lines supplied with graded dietary L-arginine.
*Journal of Animal Physiology and Animal Nutrition, submitted*

Metabolic and Clinical Response to *Escherichia coli* Lipopolysaccharide in Purebred Layer Pullets of Different Genetic Backgrounds Supplied with Graded Dietary L-Arginine.
*Poultry Science, submitted*
Furthermore, results of this thesis were presented in form of oral presentations or posters at the following conferences:

   **M.-A. Lieboldt**, I. Halle, J. Frahm und S. Dänicke

2. Establishment of an animal model of purebred laying hen genotypes diverging in production efficiency for further nutritional-physiological studies.

   *Vortragstagung – eine Gemeinschaftsveranstaltung der Gesellschaft der Förderer und Freunde für Geflügel- und Kleintierforschung e.V. (GdFuF) und des Friedrich-Loeffler-Instituts (FLI)*, 13.05.2014, Celle, Germany.


5. Influence of graded dietary L-arginine supply on growth and nitrogen balance data in female chicks of four purebred layer genotypes in early life stage.


*Vortragstagung – eine Gemeinschaftsveranstaltung der Gesellschaft der Förderer und Freunde für Geflügel- und Kleintierforschung e.V. (GdFuF) und des Friedrich-Loeffler-Instituts (FLI), 19.05.2014, Celle, Germany.*

7. Impact of different dietary L-arginine concentrations on growth of purebred laying hens of four genotypes differing in phylogeny and performance level.


*20th European Symposium on Poultry Nutrition, 24.-27.08.2015, Prague, Czech Republic, Proceedings, p. 233.*


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<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>APP</td>
<td>Acute-phase protein</td>
</tr>
<tr>
<td>APR</td>
<td>Acute-phase reaction</td>
</tr>
<tr>
<td>Arg</td>
<td>L-arginine</td>
</tr>
<tr>
<td>ASL</td>
<td>Argininosuccinate lyase</td>
</tr>
<tr>
<td>ASS</td>
<td>Argininosuccinate synthase</td>
</tr>
<tr>
<td>BH4</td>
<td>Tetrahydrobiopterin</td>
</tr>
<tr>
<td>BLA</td>
<td>High performing Rhode Island Red genotype</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>CAS</td>
<td>Chemical Abstracts Service Registry Number</td>
</tr>
<tr>
<td>CAT</td>
<td>Cationic amino acid transporter</td>
</tr>
<tr>
<td>CD14</td>
<td>Cluster of differentiation 14</td>
</tr>
<tr>
<td>Cit</td>
<td>L-citrulline</td>
</tr>
<tr>
<td>cNOS</td>
<td>Constitutive nitric oxide synthase</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cycloxygenase-2</td>
</tr>
<tr>
<td>CPS1</td>
<td>Carbamoyl-phosphate-synthase-1</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EC</td>
<td>Enzyme Commission number</td>
</tr>
<tr>
<td>E.coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>et al.</td>
<td>et aleri</td>
</tr>
<tr>
<td>FAD</td>
<td>Flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FMN</td>
<td>Flavin mononucleotide</td>
</tr>
<tr>
<td>Gly</td>
<td>L-glycine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>His</td>
<td>L-histidine</td>
</tr>
<tr>
<td>H/L-ratio</td>
<td>heterophil/lymphocyte ratio</td>
</tr>
<tr>
<td>IL-1β, IL-6, IL-8</td>
<td>Interleukin-1β, -6, -8</td>
</tr>
<tr>
<td>i.m.</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>IRF3</td>
<td>Interferon regulatory factor 3</td>
</tr>
<tr>
<td>Kdo</td>
<td>2-keto-3-deoxyoctonic acid</td>
</tr>
<tr>
<td>L68</td>
<td>Low performing New Hampshire genotype</td>
</tr>
<tr>
<td>LPB</td>
<td>Lipopolysaccharide-binding protein</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>Lys</td>
<td>L-lysine</td>
</tr>
<tr>
<td>MD-2</td>
<td>Myeloid differentiation protein-2</td>
</tr>
<tr>
<td>Met</td>
<td>L-methionine</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MyD88</td>
<td>Myeloid differentiation factor 88</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa-light-chain-enhancer of activated B cells</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>nNOS</td>
<td>Neuronal nitric oxide synthase</td>
</tr>
<tr>
<td>ODC</td>
<td>Ornithine decarboxylase</td>
</tr>
<tr>
<td>Orn</td>
<td>L-ornithine</td>
</tr>
<tr>
<td>OTC</td>
<td>Ornithine transcarbamylase</td>
</tr>
<tr>
<td>PAMP</td>
<td>Pathogen associated molecular pattern</td>
</tr>
<tr>
<td>PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Prostaglandin E2</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
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<tr>
<td>pI</td>
<td>Isoelectric point</td>
</tr>
<tr>
<td>pK_a</td>
<td>Acid dissociation constant</td>
</tr>
<tr>
<td>PRR</td>
<td>Pathogen recognition receptor</td>
</tr>
<tr>
<td>R11</td>
<td>Low performing White Leghorn genotype</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RNI</td>
<td>Reactive nitrogen intermediate</td>
</tr>
<tr>
<td>ROI</td>
<td>Reactive oxygen intermediate</td>
</tr>
<tr>
<td>TIR</td>
<td>Toll/Interleukin-1 resistance</td>
</tr>
<tr>
<td>TLR4</td>
<td>Toll-like receptor-4</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor-necrosis-factor α</td>
</tr>
<tr>
<td>TRAM</td>
<td>TRIF-related adaptor molecule</td>
</tr>
<tr>
<td>TRIF</td>
<td>TIR-domain-containing adapter-inducing interferon-β</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cells</td>
</tr>
<tr>
<td>WLA</td>
<td>High performing White Leghorn genotype</td>
</tr>
</tbody>
</table>
INTRODUCTION

To supply the steadily increasing demand for chicken products, poultry industry has changed significantly from former smallholding towards modern intensive production since the early 20\textsuperscript{th} century (ARTHUR and ALBERS 2003; WINDHORST 2006; SIEGMANN and NEUMANN 2012). In addition to the innovations in nutrition, husbandry and health management, directional genetic selection has enhanced the genetically determined performance potential of chickens and has led to higher production efficiency (HORN and SÜTÖ 2000; HAVENSTEIN et al. 2003; MATHUR 2003; RUBIN et al. 2010). However, this process has limited the genetic diversity in domestic fowl severely and has adapted breeds to a favourable environment required for implementing their genetic performance potential (GROSS 1983; HARTMANN 1990; HILLEL et al. 2003; MATHUR 2003; CHENG and MUIR 2005).

Under physiological conditions metabolic resources are appropriate, but not equally distributed between performance traits and maintenance to cope with the environment chickens are kept in (BEILHARZ et al. 1993; RAUW et al. 1998). Metabolic disorders could develop if chickens are exposed to a varying abiotic and biotic environment being not adapted to (RAUW et al. 1998). The resource allocation theory refers to those metabolic conflicts in resource allocation between performance traits and those for maintenance depending on genetically determined unbalanced use of resources for high production efficiency (GODDARD and BEILHARZ 1977; BEILHARZ et al. 1993). Perhaps selection for high genetic performance potential can be associated with a reduced adaptability of chickens to environmental alterations (VAN DER WAAIJ 2004; MIRKENA et al. 2010) and with undesirable side-effects manifesting in deficiencies of physiological, immunological and reproduction traits otherwise (MILLER et al. 1992; LIU et al. 1995; RAUW et al. 1998; VAN EERDEN et al. 2004). In relevant literature these phenomena are commonly termed as genotype-environment interactions (GROSS 1983; SHERIDAN 1990; KWAK et al. 2001; MATHUR 2003).

Among the large variety of abiotic environmental factors the adequate supply with essential nutrients such as amino acids has a major influence on the implementation of chickens’ genetically determined performance potential, health and welfare (DIETERT et al. 1994;
INTRODUCTION

Within amino acids L-arginine (Arg) is considered to be dietary indispensable in birds due to marked differences in Arg metabolism compared with mammalian species (TAMIR and RATNER 1963 a; D’MELLO 2003 a; BALL et al. 2007). Although indications for genetic differences in chicken Arg metabolism have been described in single cases (HUTT and NESHEIM 1966; AUSTIC and NESHEIM 1970; KWAK et al. 2001), there are no studies dealing with the question whether selection for high production efficiency could have reduced the adaptability of layer-type chickens to varying dietary Arg levels with regard to growth and performance traits. Furthermore, the interrelationship of Arg to several metabolic pathways is of growing interest in Arg nutrition and physiology beyond protein synthesis (WU et al. 2010; KORVER 2012). For that reason dietary Arg belongs to specific nutrients used for nutritional immunomodulation aiming at beneficial alterations in chicken’s immune response (HUMPHREY and KLASING 2004; KHAJALI and WIDEMAN 2010; KORVER 2012). In the innate immune system Arg plays a decisive role as only known precursor of multifunctional NO synthesised by cytokine or endotoxin-induced iNOS (SUNG et al. 1991; HUSSAIN and QURESHI 1997, 1998; DIL and QURESHI 2002 a, b). This fact leads to the assumption that possible selection-induced variations in chickens’ adaptability to graded dietary Arg levels could have altered Arg-depending mechanisms of avian innate immune response further. Several studies have reported on immunomodulating effects of dietary Arg in diversely immune stimulated chickens (TAYLOR et al. 1992; TAYADE et al. 2006 a, b; MUNIR et al. 2009; TAN et al. 2014), but comparatively little information is available about metabolic and immunological changes in layer-type chickens of diverse genetic backgrounds supplied with graded dietary Arg during acute-phase reaction.
1. L-arginine (Arg)

1.1 Source and occurrence

Arg is a naturally occurring and widely spread L-amino acid, bound in proteins of animal and plant origin, and can be found in physiological fluids such as blood plasma (KWAK et al. 1999; EVONIK 2010; WU et al. 2010). Proteins of total carcasses, eggs and feathers consist of 7.8, 6.4 and 7.3 % Arg in domestic fowl, respectively (NABER 1979; FISHER et al. 1981; SCOTT et al. 1982), whereas the plasma Arg concentration depends on dietary Arg intake strongly (CHU and NESHEIM 1979; KWAK et al. 1999, 2001). In poultry nutrition feedstuffs commonly used as protein sources contain larger proportions of Arg than several types of grain applied as energy sources (Table 1; EVONIK 2010).

Table 1. Proportions of crude protein, L-arginine and L-lysine analysed in feedstuffs commonly used in poultry nutrition (mean values; modified from EVONIK 2010).

<table>
<thead>
<tr>
<th>Feedstuffs (samples N)</th>
<th>Crude protein (%)</th>
<th>% in crude protein</th>
<th>% in feedstuff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Arg</td>
<td>Lys</td>
</tr>
<tr>
<td>Feather Meal (328)</td>
<td>80.3</td>
<td>6.56</td>
<td>2.60</td>
</tr>
<tr>
<td>Corn gluten meal (219)</td>
<td>60.8</td>
<td>3.14</td>
<td>1.65</td>
</tr>
<tr>
<td>Fish Meal (811)</td>
<td>59.2</td>
<td>5.61</td>
<td>6.73</td>
</tr>
<tr>
<td>Soybean Meal (1218)</td>
<td>46.9</td>
<td>7.29</td>
<td>6.12</td>
</tr>
<tr>
<td>Lupines (109)</td>
<td>36.4</td>
<td>10.85</td>
<td>4.77</td>
</tr>
<tr>
<td>Rapeseed Meal (267)</td>
<td>35.3</td>
<td>5.99</td>
<td>5.15</td>
</tr>
<tr>
<td>Beans (126)</td>
<td>25.6</td>
<td>8.78</td>
<td>6.27</td>
</tr>
<tr>
<td>Peas (138)</td>
<td>21.3</td>
<td>8.65</td>
<td>7.24</td>
</tr>
<tr>
<td>Wheat bran (173)</td>
<td>15.6</td>
<td>6.75</td>
<td>4.01</td>
</tr>
<tr>
<td>Wheat (733)</td>
<td>11.9</td>
<td>4.80</td>
<td>2.81</td>
</tr>
<tr>
<td>Triticale (177)</td>
<td>11.4</td>
<td>4.93</td>
<td>3.21</td>
</tr>
<tr>
<td>Barley (406)</td>
<td>10.8</td>
<td>4.96</td>
<td>3.61</td>
</tr>
<tr>
<td>Corn (918)</td>
<td>8.0</td>
<td>4.78</td>
<td>3.07</td>
</tr>
</tbody>
</table>

1.2 Structure and physical-chemical properties

Arg (C₆H₁₄N₄O₂, molar mass: 174.20 g/mol, CAS 74-79-3) belongs to the proteinogenic α-amino acids of which Arg contains the highest mass share of nitrogen. Its molecular structure
consists of a 4-carbon straight chain with α-carboxyl- and α-amino group at its proximal end and a complex positively charged guanidine group at its distal end (Figure 1; KHAJALI and WIDEMAN 2010; WU 2013). Due to its cationic character Arg is often found in active centres of enzymes binding phosphorylated, negatively charged ligands or in the wall of enzyme channels providing directional passing (DAMUS et al. 1973; NEMERYA et al. 1984; GALKIN et al. 2002; PRAVDA et al. 2014). The positive charge of Arg is delocalised between all three nitrogen atoms of the guanidine group in neutral, acidic and most basic environments (KHAJALI and WIDEMAN 2010; WU 2013). Arg has a $pI$ value of 10.76 and $pK_a$ values of 2.2, 9.0 and 12.5 for the α-carboxyl, α-amino and guanidine group of Arg, respectively (KHAJALI and WIDEMAN 2010; WU 2013). Based on its physical and chemical properties Arg is further classified as polar, cationic and basic amino acids such as Lys and His (D’MELLO 2003 a; WU 2013).

Figure 1. Chemical structure of L-arginine.

1.3 Metabolism in birds

1.3.1 Absorption and catabolism

While basic amino acids Lys and His are dietary indispensable for all vertebrates (POND et al. 1995; D’MELLO 2003 a), the essentiality of dietary Arg shows significant interspecies differences relating to their ability for Arg de novo synthesis (D’MELLO 2003 b; BALL et al. 2007). Dietary Arg is regarded as conditionally non-essential in most mammals depending on their developmental stage and health status (VISEK 1986; WU et al. 2010). On the contrary, Arg is essential in diets of birds gaining Arg through breakdown of body proteins and intestinal absorption (KLOSE and ALMQUIST 1940; TAMIR and RATNER 1963 a, b). Intraluminal free Arg is absorbed through the intestinal epithelial layer by sodium-dependent (e.g. $b_{0,+}$, $B_{0,+}$, $y^+$L) and high-affinity sodium independent (e.g. $y^+$) transporter systems (BRAKE and BALNAVE 1995; KANAI et al. 2000; RUEDA et al. 2003; HUMPHREY et al. 2004). Although the average standardised ileal digestibility of Arg ranges from 83 to 93 % for
most grain types and protein providing feedstuffs in pigs and chickens (STEIN et al. 2001; LEMME et al. 2004; RAVINDRAN et al. 2005; EVONIK 2010), approximately 40% of dietary Arg is already degraded by the small intestine of adult humans, rats and pigs in first pass metabolism and does not enter the systemic circulation consequently (CASTILLO et al. 1993; WU et al. 2007).

Furthermore, the primary Arg metabolism takes place in the urea cycle that exhibits significant differences between mammals and birds (Figure 2; D’MELLO 2003 b; BALL et al. 2007; WU 2013). Based on higher hepatic than renal urea cycle activity, mammals primarily catabolise Arg to Orn and urea in the liver (MORRIS et al. 1997; SHI et al. 1998), while Arg is synthesised 

\textit{de novo} from Orn and Cit in the kidney principally (WU and MORRIS 1998). The main purpose of the urea cycle is the fixation of ammonia to Orn in CPS1 (EC 6.3.4.16) and OTC (EC 2.1.3.3) catalysed reactions forming Cit (D’MELLO 2003 b; KHAJALI and WIDEMAN 2010). Afterwards Cit is transformed into Arg under the actions of ASS (EC 6.3.4.5) and ASL (EC 4.3.2.1) using amino-nitrogen of L-aspartate (BALL et al. 2007; KHAJALI and WIDEMAN 2010). In the last and most essential step urea is split off from Arg regaining Orn by the activity of arginase (EC 3.5.3.1.1; BALL et al. 2007; KHAJALI and WIDEMAN 2010). Ureotelic species like mammals require the formation of water soluble urea for disposing of excess nitrogen from protein metabolism via urinary excretion (BEQUETTE 2003).

In contrast to mammals, chickens are uricotelic organisms that excrete uric acid for nitrogen disposal in a urea cycle and Arg independent manner (EDSON et al. 1936; KARASAWA and TASAKI 1973; KARASAWA et al. 1973). This circumstance could explain the almost complete lack of urea cycle enzymes in chicken liver and the overall organ lack of mitochondrial CPS1 (Figure 2; TAMIR and RATNER 1963 a; GRAZI and MAGRI 1972; BAKER 1991; SUNG et al. 1991). However, chickens possess urea cycle remnants including arginase in their kidney where relatively low activities of OTC, ASS, and ASL are detectable (TAMIR and RATNER 1963 a; AUSTIC and NESHEIM 1970; MORRIS 2002). While ammonia fixation and Arg \textit{de novo} synthesis from dietary Orn are not possible in chickens (KLOSE and ALMQUIST 1940; TAMIR and RATNER 1963 b; GRABER and BAKER 1971), the low activities of renal ASS, ASL and arginase enable chickens to convert dietary Cit to Arg and to degrade Arg to Orn and urea further (TAMIR and RATNER 1963 a, b; SU

Figure 2. Depiction of chicken L-arginine metabolism with highlighted (X) enzymatic differences to functionally complete mammalian urea cycle (modified from SUNG et al. 1991).

1.3.2 Physiological interrelationship to further metabolic pathways

Beyond the pivotal catabolism of Arg to Orn in the presence of kidney arginase in birds and hepatic arginase in mammals (WU et al. 2010), both nitrogenous substrates are involved in diverse metabolic pathways additionally (Figure 2; D’MELLO 2003 b; KHAJALI and WIDEMAN 2010; WU et al. 2010).

First of all, Arg affects the protein metabolism either directly as primary component of body proteins (e.g. muscles, feathers, enzymes and hormones; MILLWARD and RIVERS 1988; BEQUETTE 2003) or indirectly as potent signal molecule modulating protein synthetic pathways in target cells (JEFFERSON and KIMBALL 2001; KIMBALL and JEFFERSON 2006 a, b; YAO et al. 2008; YUAN et al. 2015).

As a part of this “nutrient signalling” amino acids also sensitise target tissues to hormonal action (KUHARA 1991; NAIR and SHORT 2005) and Arg possesses potent secretagogue
activities by stimulating the release of several insulinemic (e.g. somatotropin, prolactin, IGF-1, insulin; BARBUL 1986; BRAMELD et al. 1999; SCANES 2009) and anti-insulinemic hormones (e.g. glucagon, somatostatin and catecholamines; DORSHKIND and HORSEMAN 2000; CALDER and YAQOOB 2004) in pituitary, pancreas and adrenal gland. Depending on type and amount of released hormone, Arg affects carbohydrate, protein and lipid metabolism as well as feed intake and body growth indirectly (TESSERAUD et al. 2011).

Furthermore, Arg and Orn take part in the formation of further nitrogenous metabolites. In this context the Met-dependent synthesis of creatine (KESHAVARZ and FULLER 1971 a, b; AUSTIC and NESHEIM 1972; CHAMRUSPOLLELT et al. 2002), the formation of L-proline and hydroxy-proline for collagen synthesis (AUSTIC and NESHEIM 1971; GRABER and BAKER 1973; POPOVIC et al. 2007) and that of polyamines (GRILLO et al. 1978; GRILLO 1985) are of crucial importance to chickens’ metabolism and growth performance. Polyamines are essential growth-promoting factors by maintaining membrane stability of cells (SCHUBER 1989; PIVA et al. 2002) and regulating DNA, RNA and protein synthesis as well as amino acid uptake by cells (PEGG and MCCANN 1982; SMITH 1990). The formation of polyamine follows two separate pathways: Firstly, arginine decarboxylase converts Arg to agmatine that is involved in the modulation of Arg-dependent NO synthesis as well as neurotransmission, regulation of ion channels and membrane transporters and the synthesis of polyamines (LORTIE et al. 1996; GRILLO and COLOMBATTO 2004). Secondly, putrescine derives from Orn under ODC activity and is converted to the polyamines spermidine and spermine through further inputs of Met (JÄNNE and HÖLTTÄ 1974; GRILLO 1985).

Finally, Arg plays a decisive role as the only known precursor of multifunctional NO synthesised by different NOS isoforms (MARLETTA et al. 1988; IGNARRO et al. 1993; ALDERTON et al. 2001; DAFF 2010). Latter ones transform Arg using NADPH and oxygen in a two-step reaction into Cit splitting off the short-lived free radical NO from guanidine nitrogen of Arg (Figure 3; MARLETTA et al. 1988; SUNG et al. 1991; IGNARRO et al. 1993; SU and AUSTIC 1999). This reaction requires the co-factors FAD, FMN and BH4 (ALDERTON et al. 2001) and is substrate-limited by Arg (SUNG et al. 1991; KIDD et al. 2001; RUIZ-FERIA et al. 2001). The different NOS isoforms are grouped in two classes: the constitutive NOS (cNOS) comprising the endothelial (eNOS) and neuronal NOS (nNOS), and the inducible NOS (iNOS; ALDERTON et al. 2001; DAFF 2010). While cNOS generates
intermittent low NO levels under physiological conditions (SCHMIDT et al. 1995; WU et al. 1999), iNOS has to be induced by cytokines and endotoxins to produce large quantities of NO over a prolonged period (SUNG et al. 1991; DAVIS and MATALON 2001; MURAKAMI and TRABER 2003). Depending on NOS isoforms, released NO regulates a large number of physiological processes such as vasomotory through eNOS activity in endothelial cells (MONCADA et al. 1988a, b; IGNARRO 1989; WIDEMAN et al. 1995, 1996; WU et al. 1999), neurotransmission, appetite and gut motility through nNOS activity in neurons (WIESINGER 2001; KHAN et al. 2007; WANG et al. 2014), and immune response through iNOS activity in avian thrombocytes, monocytes and macrophages (SUNG et al. 1991; KIDD et al. 2001; QURESHI 2003; BOWEN et al. 2007; ST. PAUL et al. 2012). Depending on its function in vasomotory, NO plays a pivotal role in the pathogenesis of the pulmonary hypertension syndrome in broilers (PHS; WIDEMAN et al. 1995, 1996). However, in the immune response NO primary serves as paracrine immune mediator and cytotoxic product that coordinates further immune response and kills pathogens directly (SUNG et al. 1991; KWAK et al. 2001; QURESHI 2003; BOWEN et al. 2007).

![Figure 3](image_url)

**Figure 3.** Graphical presentation of the conversion of L-arginine to L-citrulline under the action of nitric oxide synthase (NOS) splitting off multifunctional free radical nitric oxide (NO).

### 1.3.3 Immunomodulating properties of dietary L-arginine in chickens, pigs and humans

Studies on dietary Arg show a wide range of immunomodulating properties of this amino acid (SUCHNER et al. 2002; LI et al. 2007). Particularly the strong proliferative character of the cell-mediated and humoral immune response (SUCHNER et al. 2002; TONG and BARBUL 2004), and the expression of various hormone receptors within immune organs and cells provide an appropriate access to direct and indirect dietary impacts (BARBUL 1986; PLAUT 1987; DORSHKIND and HORSEMAN 2000; WINOTO and LITTMAN 2002).
To give a proper review of the findings in chicken, Table 2 summarizes the relevant studies on nutritional immunomodulation by Arg on young layer-type chicks and broilers. In addition to poultry research, enteral or even parental Arg supplementation has a crucial significance in humans suffering from severe sickness such as endotoxemia and sepsis (KIRK and BARBUL 1990). In porcine (BRUINS et al. 2000, 2002, 2003; LUIKING et al. 2005) and rodent sepsis models (NIRGIOTIS et al. 1991; MILAKOFSKY et al. 1993) it has been shown that plasma Arg availability decreases during endotoxemia and that enteral Arg supplementation is particularly advisable for the outcome of that disease (SUCHNER et al. 2002; LI et al. 2007).

In endotoxemic pigs Arg supplementation causes reduced pulmonary arterial blood pressure, improves muscle and liver protein metabolism and restored intestinal motility pattern (BRUINS et al. 2000, 2002; LUIKING et al. 2005; LUIKING et al. 2012). In humans and rodents (BARBUL 1990; KIRK and BARBUL 1990; EFRON and BARBUL 1998; EVOY et al. 1998) supplemental enteral Arg causes net nitrogen retention, increased protein synthesis and improved wound healing and tumour response on the one hand and alters cellular defence response by increasing thymic function, lymphocyte and macrophage proliferation, cytokine production as well as phagocytosis and cytotoxicity of macrophages on the other hand. Indications also exist that Arg plays a decisive role in controlling effector lymphocytes (BRONTE and ZANOVELLO 2005) and increases NO production giving rise to an impaired coagulation and vasodilation at the endothelial site subsequently (SUCHNER et al. 2002; LI et al. 2007).
Table 2. Summary of *in vivo* immunomodulating effects of dietary L-arginine on young layer-type and broiler chicks.

<table>
<thead>
<tr>
<th>Reference</th>
<th>L-arginine (% of diet)</th>
<th>Feeding duration</th>
<th>Immune stimulus</th>
<th>Bird type and breed</th>
<th>Immunomodulating effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.92/ 2.40</td>
<td>84 days</td>
<td>Rous sarcoma virus subgroup A</td>
<td>Layer (New Hamshire)</td>
<td>↑[Arg]d → ↓Mean tumor size</td>
</tr>
<tr>
<td>2</td>
<td>0.53/ 0.73/ 1.53</td>
<td>14 days</td>
<td>None</td>
<td>Layer (White Leghorn)</td>
<td>↓[Arg]d → ↓Absolute thymus, spleen and bursa weight; ↓ relative thymus and spleen weight</td>
</tr>
<tr>
<td>3</td>
<td>0.53/ 1.53</td>
<td>14 days</td>
<td><em>E. coli</em> LPS</td>
<td>Layer (White Leghorn)</td>
<td>↓[Arg]d → ↓NO production and delayed-type hypersensitivity</td>
</tr>
<tr>
<td>4</td>
<td>0.5/ 1.0/ 3.0</td>
<td>28 days</td>
<td>IBV vaccine</td>
<td>Layer (White Leghorn)</td>
<td>↑[Arg]d → ↑Absolute and relative heterophils and H:L ratio; ↓[Arg]d → ↓CD8%</td>
</tr>
<tr>
<td>5</td>
<td>1.2/ 1.5</td>
<td>16 days</td>
<td>SRBC and PHA</td>
<td>Broiler (Cobb 500)</td>
<td>↑[Arg]d → ↑Ab titer, response to PHA</td>
</tr>
<tr>
<td>6</td>
<td>Control/ control + 2.0</td>
<td>21 days</td>
<td>IBDV virulent</td>
<td>Hatchery of CARI (Izatnagar)</td>
<td>↑[Arg]d → ↑Antibody titers and mitogen of PBLs</td>
</tr>
<tr>
<td>7</td>
<td>Control/ control + 2.0</td>
<td>21 days</td>
<td>IBDV vaccine</td>
<td>Hatchery of CARI (Izatnagar)</td>
<td>↑[Arg]d → ↑Cytotoxicity and proliferation of intestinal intraepithelial lymphocytes</td>
</tr>
<tr>
<td>8</td>
<td>1.2/ 2.2</td>
<td>19 days</td>
<td>IBDV vaccine</td>
<td>Broiler (Cobb 500)</td>
<td>↑[Arg]d → ↑CD4%, CD3%, B cell%</td>
</tr>
<tr>
<td>9</td>
<td>1.0/ 1.1/ 1.3/ 1.4/ 1.5</td>
<td>21 days</td>
<td>NDV vaccine</td>
<td>Broiler (Ross x Ross)</td>
<td>↑[Arg]d → ↑Proportions of spleen, thymus and heterophils; ↑[Arg]d → ↑antibody titer</td>
</tr>
<tr>
<td>10</td>
<td>Control/ control + 2.0</td>
<td>35 days</td>
<td>HPSV virulent</td>
<td>Broiler (Hubbard x Hubbard)</td>
<td>↑[Arg]d → ↑Lymphoproliferation, cutaneous basophil hypersensitivity, lymphoid organ weight, survival</td>
</tr>
<tr>
<td>11</td>
<td>1.1/ 1.3/ 1.5</td>
<td>20 days</td>
<td>None</td>
<td>Broiler (Cobb 500)</td>
<td>↑[Arg]d → ↑Monocyte%</td>
</tr>
<tr>
<td>12</td>
<td>1.05/ 1.42/ 1.90</td>
<td>21 days</td>
<td><em>E.coli</em> LPS</td>
<td>Broiler (Ross x Ross 708)</td>
<td>↑[Arg]d → ↑Spleenic CD11+, CD14+, B cell; ↑[Arg]d → ↑Spleenic mRNA IL-1β, TLR4, PPAR-γ; ↑Cecal tonsils mRNA IL-1β, IL-10, TLR4, NF-κB</td>
</tr>
</tbody>
</table>

[Arg]d: dietary L-arginine concentration; HPSV: Hydropericardium Syndrome Virus; IBDV: Infectious Bursitis Disease Virus; IBV: Infectious Bronchitis Virus; LPS: lipopolysaccharide; NDV: Newcastle Disease Virus; PBLs: peripheral blood lymphocytes; PHA: Phytohemagglutinin; PPAR: peroxisome proliferator-activated receptor; SRBC: Sheep red blood cells

References: 1 (TAYLOR et al. 1992); 2 (KWAK et al. 1999); 3 (KWAK et al. 2001); 4 (LEE et al. 2002); 5 (ABDUKALYKOVA and RUIZ-FERIA 2006); 6 (TAYADE et al. 2006 a); 7 (TAYADE et al. 2006 b); 8 (ABDUKALYKOVA et al. 2008); 9 (JAHANIAN 2009); 10 (MUNIR et al. 2009); 11 (D'AMATO and HUMPHREY 2010); 12 (TAN et al. 2014).
1.3.4 Genetically dependent differences in L-arginine metabolism of chickens

It is generally accepted that nutrient utilization and metabolism are subjects of genetic influences (SIMOPOULOS 2002; STOVER 2006; STOVER and CAUDILL 2008). However, most studies on genetically determined differences in chicken’s Arg metabolism are from 1940s to 1970s only.

As feather proteins contain a relatively large proportion of Arg (SCOTT et al. 1982; EVONIK 2010), HEGSTEDT et al. (1941) have described a higher dietary Arg requirement for fast feathering genotypes than for slow feathering ones. Beside the rapidity of feathering, a genetically determined high expression of renal arginase increases the catabolism of Arg and leads to higher Arg requirements subsequently (NESHEIM and HUTT 1962; HUTT and NESHEIM 1966; AUSTIC and NESHEIM 1970). The renal arginase activity can be intensified by excessive dietary Lys intake further because Arg and Lys compete for renal transporters (AUSTIC and SCOTT 1975; AUSTIC and CALVERT 1981). This relationship is termed as Lys-Arg-antagonism (LATSHAW 1993; BALVANE and BRAKE 2002). For that reason, genotype-dependent variations in Lys metabolism affect Arg utilisation and requirement consequently (HUTT and NESHEIM 1966; AUSTIC and NESHEIM 1970).

Finally, genotypes requiring high dietary Arg differ from less Arg requiring genotypes in macrophage-derived NO production during innate immune response (KWAK et al. 2001).

2. Lipopolysaccharide (LPS)

2.1 Source and occurrence

Gram-negative bacteria such as Enterobacteriaceae can be found as environmental microbes in the air and litter of chicken houses ubiquitously and as commensal bacteria in chicken digestive tract (TERZICH et al. 2000; ZUCKER et al. 2000; NANDI et al. 2004). These bacteria are enveloped with an asymmetric phospholipid bilayer membrane presenting membrane-stabilising macromolecules, the so-called LPS, to their environment (HEWETT and ROTH 1993; RIETSCHEL et al. 1994). Approximately 3.5 x 10^6 LPS molecules cover three-quarter of the entire bacterial membrane surface and are released from surface in case of bacterial multiplication and death (HEWETT and ROTH 1993; RIETSCHEL et al. 1994). Free LPS acts as potent bacterial toxin, termed endotoxin, which elicits strong immune response in humans and animals (SCHLETTER et al. 1995; ADEREM and ULEVITCH
2000; CAROFF et al. 2002). LPS triggers the systemic immune response if Gram-negative bacteria such as *Escherichia coli*, *Salmonella spp.* and *Campylobacter spp.* cause systemic infections or if the intestinal absorption of LPS from gut-derived bacteria is intensified through altered intestinal barrier during certain pathophysiological conditions (HEWETT and ROTH 1993; GUARD-PETTER 2001; STERN et al. 2001; ZHAO et al. 2001; DE BUCK et al. 2004; GUERIN et al. 2010).

2.2 Chemical structure and biological properties

The LPS molecule (Figure 4) shows a common structure consisting of a hydrophilic polysaccharide region, subdivided into the O-specific chain and the core oligosaccharide, and a hydrophobic backbone termed lipid A (SCHLETTER et al. 1995; CAROFF et al. 2002). The O-specific chain presents a polymer of repeating oligosaccharide units that only appear in smooth-type Gram-negative bacteria (RIETSCHEL et al. 1994; ERRIDGE et al. 2002; RAETZ and WHITFIELD 2002). The number and structure of repeating units show large variabilities between bacteria strains by which the O-specific chain defines bacterial serotype and serves as important surface antigen (O-antigen; KNIREL and KOTCHETKOV 1994; SCHLETTER et al. 1995).

The core region of enterobacterial LPS is formally subdivided into an outer and inner portion of oligosaccharides consisting of 10 to 12 units (CAROFF et al. 2002; ERRIDGE et al. 2002). The outer core, the attachment site for the O-specific chain, exhibits intermediate structural diversity and contains hexoses D-glucose, D-galactose and N-acetyl-D-glucosamine (SCHLETTER et al. 1995; CAROFF et al. 2002; RAETZ and WHITFIELD 2002). The inner core serves as linkage to the lipid A region and comprises units of the acidic sugar 2-keto-3-deoxyoctonic acid and L-glycero-D-manno configured heptose mainly (RAETZ and WHITFIELD 2002). Both saccharide types are generally substituted by negatively charged phosphate groups stabilising core’s structure by binding divalent cations (RIETSCHEL et al. 1994; RAETZ and WHITFIELD 2002). This region exhibits low diversity in its structure only (SCHLETTER et al. 1995).

Finally, the lipid A region acts as hydrophobic, covalently bound LPS anchor in the outer bacterial membrane (RIETSCHEL et al. 1994; RAETZ and WHITFIELD 2002). Lipid A is a phospholipid that consists of glucosamines and several hydrophobic saturated fatty acids that
vary in their number, location and chain length (GALANOS et al. 1985; ZÄHRINGER et al. 1994). Lipid A together with bound inner core is the minimal LPS structure required for bacterial viability (SCHLETTER et al. 1995; ALEXANDER and RIETSCHEL 2001) and therefore a highly conserved structure (LÜDERITZ et al. 1973; CAROFF and KARIBIAN 2003), mainly responsible for the endotoxic activity and immunomodulating properties of LPS (ZÄHRINGER et al. 1994; RAETZ et al. 2006).

![Figure 4](image-url). The common chemical structure of LPS of Enterobacteriaceae (adapted from SCHLETTER et al. 1995; GlcN: glucosamine; Hep: L-glycero-D-manno-heptose, Kdo: 2-keto-3-deoxyoctulosonic acid, P: phosphate; zig-zag lines: fatty acids).

2.3 Mode of action

2.3.1 Recognition by avian innate immune system

Entering hosts through environmentally exposed surfaces Gram-negative bacteria activate tissue resident sentinel cells of host’s innate immune system (QURESHI 2003; KANNAKI et al. 2010). These cells present specific PRR detecting bacteria by means of conserved antigen molecules, termed PAMPs, such as LPS (MEDZHITOV and JANEWAY 1997a, b; AKIRA 2004). In vertebrates the TLR4 shows highly conserved ligand specificity to bacterial LPS (LEVEQUE et al. 2003; AKIRA et al. 2006; KANNAKI et al. 2010). In chickens TLR4 is detected on the surface of antigen presenting cells such as macrophages and monocytes as well as on heterophils, thrombocytes and cells of almost every internal organ (FARNELL et al. 2003; IQBAL et al. 2005; HIGGS et al. 2006; FERDOUS et al. 2008). The successful activation of TLR4 requires the preceding association of LPS with chicken LPS binding
protein (LBP; JUUL-MADSEN et al. 2014) and its consecutive attachment to the LPS-binding complex consisting of TLR4 and extracellular co-receptors CD14 and MD-2 (Figure 5; WRIGHT et al. 1990; POLTORAK et al. 1998; KOGUT et al. 2005; PARK et al. 2009).

**2.3.2 Intracellular signalling in TLR4/MD-2 expressing cells**

Based on its transmembrane character TLR4 exhibits an extracellular, ligand-binding domain and an intracellular, signal transducing domain (ROCK et al. 1998; RALLABHANDI et al. 2006; JIN and LEE 2008). When LPS attaches to LPS-binding complex, MD-2 alters the conformation of TLR4 and enables a direct contact between TLR4 and LPS (MEDZHITOV et al. 1997; POLTORAK et al. 2000; VISINTIN et al. 2003; PARK et al. 2009). Two major intracellular signal cascades exist in mammalian TLR4-expressing cells (Figure 5): the early MyD88-dependent and the delayed MyD88-independent response (HOEBE et al. 2003; YAMAMOTO et al. 2003). Including various sequences of adaptor proteins and signalling intermediates, the MyD88 dependent pathway activates the transcription factors NF-κB ultimately (GENG et al. 1993; TAKEDA and AKIRA 2004; HAN et al. 2009), whereas the MyD88-independent pathway induces IFN regulatory factor 3 (IRF3) and a delayed NF-κB response (AKIRA 2001; DOYLE et al. 2002; ZHAI et al. 2004). However, chickens lack the MyD88-independent, TRAM/TRIF-mediated pathway in TLR4-expressing cells (KEESTRA and VAN PUTTEN 2008), which could explain chicken’s higher resistance to LPS compared with mammals (KANNAKI et al. 2010).

In the following the activated transcription factor NF-κB translocates to the nucleus and induces the expression of genes encoding avian equivalents of mammalian pro-inflammatory cytokines IL-1β, IL-6 and IL-8, and COX-2 and iNOS (MEDZHITOV and JANENAY 2000; DIL and QURESHI 2002 a; TAKEDA and AKIRA 2005; TOMAS-COBOS et al. 2008; HE et al. 2006). Although TNF-α-like activities have been observed in chickens, the gene encoding TNF-α has not been found in chickens recently (KAISER et al. 2005; KAISER and STÄHELI 2014). On the contrary, IL-1β (WEINING et al. 1998; GYORFY et al. 2003) and IL-6 (SCHNEIDER et al. 2001; SMITH et al. 2005) have been clearly identified in chicken. After new gene transcription cytokines and enzyme products are released and coordinated early acute-phase reaction (XIE et al. 2000; GRUYS et al. 2005).
2.3.3 **Genetically determined differences in the interaction of LPS and TLR4 in chickens**

Susceptibility and resistance to diseases as well as the associated immune response can vary between chicken lines (ZEKARIAS et al. 2002). For instance, the resistance to *Salmonella enterica* serovar *Typhimurium* is closely associated with the location of TLR4 gene in chicken genome (DIL and QURESHI 2002 a; LEVEQUE et al. 2003; MALEK et al. 2004). In this context, genotype-depending variations in absolute and relative TLR4 expression on macrophages and causal TLR4 gene polymorphisms are also described (DIL and QURESHI 2002 a; MALEK et al. 2004; ABASHT et al. 2009). Based on higher CD14 and TLR4 expressions on the macrophage’s surface (DIL and QURESHI 2002 a, b), chicken genotypes can be classified in hyper-responders and hypo-responders for iNOS expression (HUSSAIN and QURESHI 1997, 1998).

Furthermore, non-selected chicken lines respond stronger to *Salmonella* infections by higher mRNA expression of pro-inflammatory and anti-inflammatory cytokines’ in heterophils than selected meat-type and layer-type chicken lines (REDMOND et al. 2009). Heterophils’
immune function also depends on genotype-specific rapidity in feathering (SWAGGERTY et al. 2003 a, b) since heterophils of fast feathering genotypes show more effective phagocytosis and killing of bacteria as well as stronger degranulation, oxidative burst and higher initial values of IL-6 and IL-8 than those of slow feathering ones (SWAGGERTY et al. 2003 b).

2.4 The LPS-induced acute-phase reaction and its associated systemically metabolic, clinical and immunological alterations

The acute-phase reaction (APR) is an early, non-specific, non-adaptive defence response of the innate immune system aiming at rapid re-establishment of homeostasis (GRUYS et al. 2005; O’REILLY and ECKERSALL 2014). Depending on their severity, APR induction such as bacterial infection, trauma, neoplasms and other lesions causing cell damage can enlarge the early local immune response to a systemic event with marked metabolic, endocrine and immunological alterations (CHAMANZA et al. 1999; HUMPHREY and KLASING 2004; GRUYS et al. 2005). In poultry the APR can be induced by local or systemic LPS application experimentally (XIE et al. 2000; CHENG et al. 2004; SHINI et al. 2008; TAN et al. 2014).

At the site of lesion tissue resident sentinel cells such as macrophages and dendritic cells are activated through the interaction of PRR and PAMP that cause the release of diverse mediators (VAN MIERT 1995; MEDZHITOV and JANEWAY 2000; TOMAS-COBOS et al. 2008; HE et al. 2006). These molecules include vasoactive substances (e.g. histamine, prostaglandins, leukotrienes, NO), pain promoting mediators (e.g. bradykinin, serotonin, PGE₂) as well as pro-inflammatory cytokines (IL-1β, IL-6, TNF-α-like), chemokines (e.g. IL-8) and pathogens directly killing RNI and ROI (TIZARD 2009; MURPHEY 2012; JUUL-MADSEN et al. 2014). In particular, chemokines are of crucial importance for attracting further leukocytes from peripheral blood and lymphoid organs to enter the infected tissue via diapedesis and sustain inflammatory response (BAGGIOLINI 1998; LUSTER et al. 1998).

In order to facilitate diapedesis, released vasoactive molecules and TNF-α activate endothelial cells to express adhesion molecules (e.g. selectins), dilate blood vessels, slow blood flow down and increase endothelial permeability (STRIETER et al. 1989; LAMAS et al. 1991; HARRIS et al. 2002). Thereafter, plasma components (e.g. complement factors) and cells (e.g. leukocytes, thrombocytes, and erythrocytes) leave the blood, enter the site of infection and trigger inflammatory response (GRUYS et al. 2005; JUUL-MADSEN et al. 2014).
complement factors improve phagocytosis via opsonising bacteria, perform chemotaxis and direct cytolysis of bacteria as well as they enhance T and B cell immunity (HARMON 1998; CARROLL 2004; RUS et al. 2005).

As a result of early inflammatory response, severe alterations in peripheral leukocyte proportions occur. During the first 24 hours, systemic leukopenia comprising heterophilia, lymphopenia and thrombocytopenia occurs, peaks at 12 hours after induction and changes to a leukocytosis associated with lymphocytosis and monocytosis at 48 to 72 hours (GROSS and SIEGEL 1983; WANG et al. 2003; SHINI et al. 2008; BOWEN et al. 2009).

During the first 6 to 12 hours, heterophils form the first line defence in chickens since they are attracted to the site of infection at first (ANDREASEN et al. 1993; HARMON 1998; GENOVESE et al. 2013). In contrast to mammalian neutrophils, chicken heterophils lack catalase and myeloperoxidase required for a sufficient oxidative burst (HARMON 1998; GENOVESE et al. 2013). However, these cells kill bacteria effectively by phagocytosis, degranulation and the synthesis of lysozyme and different kinds of antimicrobial peptides (e.g. cathelicidin-like proteins, defensins; VAN DIJK et al. 2009; GENOVESE et al. 2013).

A further difference to mammalian immune response is formed by chicken thrombocytes, which act as important immune cells supporting innate immune response by phagocytosing bacteria and releasing pro-inflammatory cytokines IL-1β and IL-6 as well as PGE₂ and NO after TLR4 activation (FERDOUS et al. 2008; SCOTT and OWENS 2008; ST. PAUL et al. 2012). After heterophils and thrombocytes, blood monocytes also enter the site of infection, differentiate into macrophages and sustain inflammation further by chemotaxis, phagocytosis, killing bacteria and pro-inflammatory cytokine, NO and PGE₂ production (QURESHI et al. 2000; QURESHI 2003; JUUL-MADSEN et al. 2014).

In case of severe APR induction or prolonged exposure to pathogens, a large amount of pro-inflammatory cytokines enter the blood circulation and induce severe metabolic, endocrine and immunological alterations systemically (BESEDOVSKY and DEL REY 1997, 2001; WIGLEY and KAISER 2003; GRUYS et al. 2005). This process is accompanied by the gradual transition from innate to acquired immunity implemented by the cell-mediated (T and B lymphocytes) and humoral immune response (JUUL-MADSEN et al. 2014). The majority of such systemic alterations results from cytokines’ action on the hypothalamus and its subordinated hypothalamic-pituitary-adrenal axis (CURTIS et al. 1980; KLASING and

As pro-inflammatory cytokines are rapidly cleared from circulation, the APR can be sustained over a prolonged time by specific plasma proteins, the so-called acute-phase proteins (APP; CHAMANZA et al. 1999; GRUYS et al. 2005). For that reason, hepatocytes’ protein synthesis and secretion of specific plasma proteins is activated by released IL-6 directly and hypothalamic-pituitary-adrenal-axis through glucocorticoids indirectly (AMRANI et al. 1986; KLASING and JOHNSTONE 1991). The APP can increase or decrease in their concentration and are termed positive or negative APP, respectively (KLASING and JOHNSTONE 1991; GRUYS et al. 1994; GRUYS and LANDMAN 1997). While the concentration of albumin, the major negative APP in chicken, decreases to 50 to 75 % of its physiological baseline (ADLER et al. 2001), the concentration of positive APP increases up to 2 to 1000 fold of their basal levels depending on the class of positive APP (GRUYS et al. 2005). These classes are: minor (e.g. ceruloplasmin, fibrinogen; peak at 24 to 72 hours), moderate (e.g. α1-acid-glycoprotein; peak at 24 to 48 hours) and major (e.g. serum amyloid A; peak at 7 to 10 days) positive APP (CHAMANZA et al. 1999; O’REILLY and ECKERSALL 2014). The functions of APP comprise opsonisation of microbes, chemotaxis of leukocytes and binding plasma cooper, iron and zinc ions to prevent their uptake by microbes (LAURIN and KLASING 1987, TAKAHASHI et al. 1997; WEINBERG 1999).

In addition to immunological changes, the sickness-induced anorexia and metabolic activation of the immune system further dysregulate metabolic homeostasis during early inflammation (ELSASSER et al. 2000; HUMPHREY et al. 2002). The maintenance of certain metabolic pathways and the sufficient realization of fever response require adequate amounts of energy from lipid and protein breakdown (BARACOS et al. 1987; CHIOLÉRO et al. 1997). As the synthesis of protective factors (e.g. complement, APP, cytokines, antibodies) and leukocyte proliferation need increased quantities of amino acids (KLASING and AUSTIC 1984; DAHN et al. 1995; BIOLO et al. 1997), especially aromatic ones (REEDS et al. 1994), the uptake of amino acids by the liver increases severely, in lymphoid organs moderately and decreases in
skeletal muscular strongly (BARNES et al. 2002; HUMPHREY and KLASING 2004). The cytokine-induced anorexia restricts the dietary input of amino acids further and makes endogenous protein breakdown necessary (KLASING and AUSTIC 1984; KLASING 1988). As skeletal muscles represents the largest labile pool of amino acids (HENTGES et al. 1984; TIAN and BARACOS 1989), TNF-α coordinates in mammals the required protein degradation and following release of amino acids from skeletal muscles and the reduced hepatic synthesis of negative APPs (ROSENBLATT et al. 1983; SAX et al. 1988; COONEY et al. 1997; GRUYS et al. 2005). As a result of these severe alterations in protein and amino acid metabolism, the whole body nitrogen balance expresses strong changes (BIOLO et al. 1997; BREUILLE et al. 1999; DICKERSON et al. 2001; BRUINS et al. 2002). In chronic stages of inflammation, IL-1β-induced anorexia and TNF-α-induced protein catabolism can lead to severe cachexia well visible by declines in growth rate and protein accretion (BENSON et al. 1993; WEBEL et al. 1998).
SCOPE OF THE THESIS

The present thesis aimed to test the following hypotheses derived from scientific literature referred to in introduction and background:

1. A marginal dietary Arg supply induces adaptive difficulties in growth and laying performance which are less pronounced in genetically low performing layer-type chickens than in high performing ones.

2. The metabolic and immunological response of genetically high performing layer-type chickens to a LPS-induced acute-phase reaction is more pronounced by a dietary Arg supply beyond the requirement for optimal growth and performance when compared to low performing strains.

These hypotheses were tested using a chicken model (Figure 6) that contrasted four purebred layer lines differing in their performance level (high vs. low) and phylogenetic origin (white vs. brown layer) each. In a first step genotypes’ suitability for investigating both hypotheses named above were verified by characterising female chickens of these genotypes in their growth and performance potential from hatch to the 74th week of age under commercial feeding conditions (Paper I).

![Figure 6. Depiction of the established chicken model contrasting four different genotypes.](image)

In a second step two experiments were conducted to examine genotypes’ adaptability and sensibility to varying concentrations of dietary Arg. For that reason, female chickens of each
genotype were long-termly supplied with graded dietary Arg equivalent to 70, 100 and 200 % of recommended age-dependent Arg level (NRC 1994). From hatch to the 41st week of age the effects of dietary Arg on growth and performance traits of genotypes were investigated (Paper II). In a parallel study female chickens of each genotype were reared under the same conditions described in Paper II to examine the effects of dietary Arg on growth of internal organs from hatch to the 18th week of age (Paper III).

Furthermore, in two separate experiments the interacting effects of a long-term graded dietary Arg supply and a single E.coli LPS injection on the metabolic, clinical and immune response in chickens of different genetic backgrounds were studied until 48 hours post-injection. Therefore, 12-week-old cockerels (Paper IV) and 18-week-old pullets (Paper V) reared under the same conditions, described in the preceding studies (Paper II and III), were one-time treated with 2 mg LPS/ kg BW i.m.. In differently Arg supplied cockerels the LPS-induced alterations in haematology, body temperature, feed intake and weight gain were investigated until 48 hours post-injection (Paper IV). Additionally, the LPS-induced changes in metabolic and clinical response were closer studied in differently Arg supplied pullets by examining nitrogen balance, plasma amino acids, clinical scoring and core body temperature until 48 hours post-injection (Paper V).

The sequential course of studies carried out in this thesis is given in Figure 7.

**Figure 7.** Schematic presentation of studies carried out in the present thesis (I – V: Paper of the related studies).
Phylogenic versus selection effects on growth development, egg laying and egg quality in purebred laying hens

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European Poultry Science (EPS)

Volume 79
DOI: 10.1399/eps.2015.89

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Summary

The aim of the present study was to assess the performance traits of chicken lines with different performance level and phylogenetic origin. Selection for high performances may change unselected traits related to animal health and well-being. However, long before intense selection started to act leading to contemporary high performing genotypes, founder populations on egg laying breeds with white and brown egg shell had been separated for many generations and have evolved independently. We have started to set up a comprehensive collaborative effort at the Friedrich-Loeffler-Institut to approach research question related to the capacity of high selected chicken lines to cope with limited metabolic resources. As a first step, four genotypes of purebred laying hens (WLA, BLA, R11 and L68) were used, which were divided by their divergence in performance and phylogeny. For the first time these genotypes were characterized according to their performance and growth development in the first 16 weeks of age in a rearing trial, a pre-laying period of 6 weeks and a following performance trial of 13 laying months (23rd to 74th week of age). The investigated performance traits were significantly affected by genotype, age and their interaction (p ≤ 0.001). As a result of selection for high laying performance, selected strains showed a significantly higher performance than the non-selected ones. The high performing genotypes had an average laying intensity of 85 to 90 %, a daily egg mass production of approximately 50 g/hen/d and a feed to egg mass ratio of 2.1 to 2.3 kg/kg. However, the low performing genotypes had an average laying intensity of 52 to 56 %, a daily egg mass production of approximately 26 to 31 g/hen/d and a feed to egg mass ratio of approximately 3.0 kg/kg. Concerning average egg weight only R11 (50 g/egg) differed from the other experimental lines (55 to 58 g/egg). Independently of their performance brown hens showed a significantly higher body weight than white hens during the whole trial.

Egg quality analyses showed that high performing lines had a significantly higher albumen proportion (57.1 to 62.4 %) and a significantly lower yolk proportion (26.8 to 29.8 %) than the low performing lines (albumen: 55.3 to 57.4 %, yolk: 30.3 to 33.5 %). White hens (10.8 to 13.6 %) had significantly higher proportion of egg shells than brown hens (10.3 to 12.9 %).

In summary, the studied genotypes showed clear differences in performance level that made them well suitable for the established experimental design. With that design further studies should be carried out under varying environmental conditions (e.g. feeding, housing,
infectious diseases). Thereby the studies will examine the question whether selection on high performance (WLA, BLA) leads to a reduced adaptability to varying environmental conditions.

**Keywords:** experimental design, chicks, pullets, laying hens, genotypes, growth, laying performance, egg quality, Gompertz equation

**Introduction**

Efficiency of poultry production is affected by several factors like feed costs, animal health and welfare, and a wide range of environmental conditions (YALCIN et al. 2005; DARMANI KUHI et al. 2010). Main objectives in breeding of laying hens are to achieve a large number of saleable eggs, great persistency in laying performance, good inner and outer egg quality and a low feed to egg mass ratio. In addition, efforts have been made to improve health and therefore welfare, and to guarantee a good adaptation to different kinds of housing systems (PREISINGER 2012). Due to efficient selection the egg production has grown dynamically, and the world’s annual egg production is estimated to be 1284 million (FAO 2014). HORN and SÜTÖ (2000) demonstrated that the breeding process of the last two decades of the 20th century improved the egg production of white layers by two eggs per year. The poultry market of today is dominated by only a few breeding companies worldwide, whereas about 100 years ago nearly 40 chicken breeds were used in breeding stations in Germany (KNISPEL 1908). World’s egg consumption is covered to 50 % by white egg layer hybrids (HORN and SÜTÖ 2000), which have been derived from one single breed, the White Leghorn (CRAWFORD 1990).

While directional genetic selection is the major contributor to the changes in performance potential (HAVENSTEIN et al. 2003), it has been reported that selection for high production efficiency in livestock species is associated with undesirable side-effects such as deficiencies in physiological, immunological and reproduction traits as well as behavioral problems (DUNNINGTON 1990; MILLER et al. 1992; LIU et al. 1995; RAUW et al. 1998). Such undesirable side-effects might be related to an imbalance in resource allocation (GODDARD and BEILHARZ 1977). Due to adaptation of genotypes, the metabolic resources used by an
animal should be optimally distributed between maintenance to cope with the environment in which they are kept, and production traits (BEILHARZ et al. 1993). As selection aims at minimizing the metabolic resources not needed for maintenance, VAN DER WAAIJ (2004) and MIRKENA et al. (2010) hypothesized that high performing genotypes have a reduced capacity to compensate unexpected environmental changes like limited resources compared to low performing genotypes. To approach this hypothesis we have started a comprehensive collaboration at the Friedrich-Loeffler-Institute to study the effect of selection on performance efficiency towards the adaptability of laying hens under varying environmental conditions in a phylogenetic context. The design of this ongoing research activity is formed by four purebred layer lines differing in performance level and phylogenetic origin (Fig. 1). Two high performing, commercial genotypes (WLA and BLA) taken from breeding program of Lohmann Tierzucht GmbH are contrasted to two low performing ones (R11 and L68). R11 and L68 chicken lines are maintained as non-selected resource populations at the Institute of Farm Animal Genetics, Friedrich-Loeffler-Institute, at Mariensee. The line R originated from the Cornell Line K (COLE and HUTT 1973), and has been introduced to the Institute in the 1960s (HARTMANN 1987). Line L68 is a New Hampshire line, which was founded in the 1970s in the former German Democratic Republic (VEG Vogelsang). The two white layer lines (WLA and R11) are of White Leghorn origin and phylogenetically closely related, but distant from the Rhode Island Red higher performing line (BLA) and its low performing counterpart L68 (LYIMO et al. 2014).

As a first study, we report here on the characterization of the experimental model of four chicken lines towards the effects of phylogeny and selection on growth and laying performance from hatch to the end of the 74th week of age.

**Material and Methods**

*Rearing trial*

After hatch a total of 516 one day-old female chicks were housed over a period of 16 weeks in a floor-range system. Due to diverging hatch results of the different genotypes (data not shown) the number of housed day-old chicks varied between the four genotypes (140 chicks of WLA, 76 chicks of BLA, 147 chicks of R11 and 153 chicks of L68). Light was provided for 24 hours on day 1-2. From day 3 onwards light was reduced to 15 hours in the first week
From week 1 to 7 light period was reduced stepwise by one hour a week to 9 hours and maintained until the end of rearing (16th week of age). Temperature programme followed usual specifications and the animals were vaccinated against MD, ND and IB. After hatch every chick was equipped with an individual wing-tag, and genotypes were placed separately to a single compartment of a floor-range system with nipple drinkers and a feeding trough. During the whole trial feed and water were provided ad libitum. Chicks were fed with a commercial grain-soybean meal diet (Table 1) from week one to seven (approx. 170 g crude protein and 11.5 MJ AME_N/kg diet). From week eight to 16 growing pullets were also fed with a commercial grain-soybean meal diet (approx. 135 g crude protein and 11.3 MJ AME_N/kg diet). Diets were formulated to meet nutrient requirements according to the recommendations of the National Research Council (NRC 1994) and Society of Nutrition Physiology (GFE 1999).

In the first half of the rearing trial (hatch to eighth week of age) the animals were weighed once a week, while in the second half (eighth to 16th week of age) they were weighed every second week. Feed not consumed was recorded weekly. The daily weight gain and the feed to gain ratio were calculated.

**Figure 1.** Experimental design of purebred laying hens differing in performance level and phylogenetic relationship.

Mit Versuchsdesign von Reinzuchtlegehennen unterschiedlichen Leistungs niveaus und phylogenetischer Verwandtschaft.

**Performance trial of laying hens**

At the end of the rearing trial, 192 17-week-old pullets (48 of each genotype) were moved to a layer facility with single cages in a three-floor cage system in random order. Each genotype was allocated to one experimental group. The single cages enabled individual records of
laying performance and feed intake. Each cage (50 cm x 46 cm x 43 cm) was equipped with a feeding trough, a nipple drinker and a perch. Feed and water were provided *ad libitum*. From 17\textsuperscript{th} week of age the light duration was increased by half an hour per week to 14 hours of light at 23\textsuperscript{rd} week of age.

After a pre-laying period from week 17 to 22, the performance trial was subdivided into thirteen 28-day laying periods. The trial ended at week 74. Hens were fed with a commercial grain-soybean meal diet (approx. 150 g crude protein and 10.6 MJ AME\textsubscript{N}/kg diet; Table 1). The diets were formulated to meet nutrient requirements according to the NRC (1994) and GFE (1999) recommendations for high performing laying hens.

Hens were weighed at the end of every 28-day laying period. Eggs were recorded daily. Defective eggs (shell-less, cracked, double eggs) were also recorded. For each laying period the egg weight was monitored by collecting all laid eggs of each hen on three consecutive days in a two-week interval. Feed not consumed was recorded weekly. Based on the feed intake and egg mass the feed to egg mass ratio was calculated.

*Egg quality parameters*

In the 40\textsuperscript{th}, 65\textsuperscript{th} and 74\textsuperscript{th} week of age eggs of each hen were collected on three consecutive days (40\textsuperscript{th} week: 416 eggs, 65\textsuperscript{th} week: 328 eggs, 74\textsuperscript{th} week: 250 eggs). Eggs were weighed and egg yolk and albumen were separated. Weight of the shell, including the inner shell membrane, and weight of yolk were recorded. The weight of albumen was determined by subtracting yolk and shell weight from the original egg weight; yolk to albumen ratio was calculated. Weights of the egg components are presented in percentage as proportions of the whole egg weight. Yolk color was estimated by using a Roche-fan (15 fans, F. Hoffmann-La Roche Ltd., Basel, Switzerland).

*Dry matter and crude nutrients of feed*

Diets (Table 1) were analyzed for dry matter, crude ash, crude fat, crude fiber, neutral and acid detergent fiber, starch, sucrose, phosphorous, calcium and Kjeldahl N according to the methods of the Association of German Agricultural Analytic and Research Institutes (VDLUFA; BASSLER 1993). Crude protein of the diets was calculated by multiplying the Kjeldahl N by 6.25. The apparent metabolizable energy concentrations corrected to zero
nitrogen balance (AME_N) of the diets were calculated according to the energy estimation equation of the World’s Poultry Science Association (VOGT 1986).

Table 1. Composition, calculated and analyzed nutrient contents of the experimental diets.
Zusammensetzung, kalkulierte und analysierte Inhaltsstoffe der Versuchsrationen.

<table>
<thead>
<tr>
<th>Ingredients, g/kg diet</th>
<th>Chicks (week 1-7)</th>
<th>Pullets (week 8-16)</th>
<th>Layers (week 17-74)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>235.6</td>
<td>-</td>
<td>49.9</td>
</tr>
<tr>
<td>Wheat</td>
<td>200.0</td>
<td>389.2</td>
<td>470.0</td>
</tr>
<tr>
<td>Barley</td>
<td>200.0</td>
<td>300.0</td>
<td>200.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>165.0</td>
<td>100.0</td>
<td>159.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>2.0</td>
<td>2.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Field Peas</td>
<td>100.0</td>
<td>120.0</td>
<td>-</td>
</tr>
<tr>
<td>Lucerne pellets</td>
<td>50.0</td>
<td>50.0</td>
<td>-</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>22.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>15.0</td>
<td>10.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>-</td>
<td>16.0</td>
<td>92.5</td>
</tr>
<tr>
<td>Premix 1</td>
<td>9.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Premix 2</td>
<td>-</td>
<td>10.0</td>
<td>-</td>
</tr>
<tr>
<td>Premix 3</td>
<td>-</td>
<td>-</td>
<td>10.0</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.4</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>-</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Anticoccidal (Sacox 12%)</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Chemical composition, g/kg dry matter

| Dry matter  | 888.1 ± 0.4 | 883.6 ± 0.6 | 911.7 ± 0.5 |
| Crude ash   | 50.45 ± 0.83| 67.53 ± 0.85| 152.41 ± 3.57|
| Crude protein| 189.61 ± 1.17| 151.67 ± 2.41| 168.11 ± 1.61|
| Crude fat   | 31.38 ± 0.58| 30.21 ± 1.43| 29.43 ± 0.77 |
| Crude fiber | 53.33 ± 1.57| 45.91 ± 1.72| 30.55 ± 0.19 |
| Neutral detergent fiber| 223.26 ± 13.73| 186.20 ± 4.41| 161.13 ± 8.70|
| Acid detergent fiber| 67.46 ± 1.09| 57.09 ± 4.00| 50.11 ± 5.99 |
| Starch      | 501.69 ± 1.00| 538.24 ± 2.42| 459.06 ± 2.07 |
| Sucrose     | 44.51 ± 0.05| 34.45 ± 0.27| 30.55 ± 0.17 |
| Phosphorous | 6.94 ± 0.28 | 8.11 ± 0.29 | 5.06 ± 0.21 |
| Calcium     | 9.14 ± 0.36 | 15.83 ± 0.44 | 50.05 ± 0.81 |
| AME_N (MJ/kg DM) | 12.97 ± 0.05 | 12.82 ± 0.06 | 11.68 ± 0.05 |
| Methionine  | 3.23           | 2.77           | 2.82               |
| Lysine      | 9.91           | 8.45           | 7.87               |

1 premix – chicks: feed additives (per kg premix): Vitamin A, 1,200,000 IU; Vitamin D₃, 350,000 IU; Vitamin E, 4,000 mg; Vitamin B₂, 250 mg; Vitamin B₆, 800 mg; Vitamin B₁₂, 600 mg; Vitamin B₁₂, 3,200 µg; Vitamin K₃, 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, 8,000 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 D₃, 200,000 IU; Vitamin E, 2,500 mg; Vitamin B₂, 500 mg; Vitamin B₆, 400 mg; Vitamin B₁₂, 1,850 µg; Vitamin K₃, 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 premix – hens: feed additives (per kg premix): Vitamin A, 1,000,000 IU; Vitamin D₃, 250,000 IU; Vitamin E, 2,000 mg; Vitamin B₂, 250 mg; Vitamin B₆, 700 mg; Vitamin B₁₂, 400 mg; Vitamin B₁₂, 2,000 µg; Vitamin K₃, 400 mg; Nicotin amide, 4,000 mg; Calcium-D-pantothenate, 1,000 mg; Folic acid, 60 mg; Biotin, 2,500 µg; Choline chloride, 40,000 mg; Fe, 4,000 mg; Cu, 1,000 mg; Mn, 10,000 mg; Zn, 8,000 mg; I, 120 mg; Se, 25 mg; Co, 20.5 mg; Butylated hydroxy toluene (BHT), 12,500 mg; Beta-carotene, 400 mg; Canthaxanthin, 400 mg
4 analyzed
5 apparent metabolizable energy concentrations corrected to zero nitrogen balance (AME_N), calculated according to the energy estimation equation of the WPSA (VOGT 1986)
6 calculated
Modelling of growth curves

The time-dependent individually recorded growth data (cumulative growth; \( n = 48 \) per genotype) were fitted to the growth function of GOMPertz (1825) regressively from hatch to the end of the 74\(^{th}\) week of age. That data were analyzed by means of the procedure “nonlinear regression” of the software package “Statistica 10.0 for the Windows\(^{TM}\) Operating System” (STATSOFT INC. 2011). The method of parameter estimation was calculated using the iterative Quasi-Newton method.

\[
y(t) = a \cdot e^{-b \cdot e^{-ct}}
\]

Where \( y(t) \) = body weight (g) of the hen at time \( t \), expressed as a function of \( a \); \( a \) = adult body weight (g) of the hen (asymptotic limit); \( b, c \) = parameters of the function (regression coefficients); and \( t \) = time (weeks) taken to reach the maximum rate of maturity.

The age at maximum body weight gain (\( t_{\text{max}} \)), that is equivalent to the point of inflection of the cumulative, sigmoid growth curve, was calculated by the second derivative of the cumulative growth function:

\[
t_{\text{max}} = \frac{\ln b}{c}
\]

The maximum daily weight gain was computed by substituting the genotype specific calculated \( t_{\text{max}} \) in the derivative of the cumulative growth function of the associated genotype.

Statistical analyses

Statistical analysis of performance traits was carried out by means of a two factorial analysis of variance (ANOVA) with genotype, age and their interaction as fixed effects. For traits measured repeatedly on the same animal (e.g. body weight, feed intake and egg weight) a “repeated” statement was considered in the statistical model to account for similarities within subjects. Statistical analysis of calculated growth function parameters was carried out by means of a one factorial ANOVA with genotype as fixed effect. In both cases the Tukey-Kramer test was applied for a multiple comparison of means. Data were reported as least square mean values and standard error. Differences between genotypes were considered to be statistically significant for \( p < 0.05 \). ANOVA of performance traits was performed using the procedure MIXED and ANOVA of calculated growth function parameters was performed using procedure ANOVA of the software package SAS 9.2 (SAS INSTITUTE INC. 2010).
Results

Rearing trial

During the 16 weeks rearing trial genotype, age and their interaction affected body weight, daily weight gain, daily feed intake and feed to gain ratio significantly ($p < 0.001$; Table 2). Body weight of different genotypes showed a time-dependent increase ($p < 0.001$) over the 16 weeks trial. The mean hatch weight (32 to 38 g/chick) did not differ statistically between the genotypes. After 16 weeks L68 achieved the highest body weight (1249 g/chick) of the four genotypes ($p < 0.05$). The high performing BLA (1180 g/chick) and WLA (1107 g/chick) differed significantly from each other, while R11 had the lowest body weight (854 g/chick) after 16 weeks. From week four onwards L68 started to differ significantly from the other genotypes and line R11 showed the lowest body weight ($p < 0.05$). Until the end of the trial the high performing genotypes did not differ from each other.

According to the development of body weight, genotype also significantly influenced daily weight gain ($p < 0.001$; Table 2). All genotypes showed highest daily weight gain at the tenth week of age ($p < 0.05$). The highest weight gain of 14.5 g/chick/d was recorded in line L68 from week nine to twelve. In the first half of the trial line L68 differed significantly from the other genotypes and WLA and BLA did not differ from each other. During the entire rearing R11 line achieved lowest daily weight gain of all four lines. In the second half both brown lines did not differ from each other. Age also affected this trait high significantly ($p < 0.001$), as daily weight gain increased until week 10, and then strongly decreased to the end of trial.

Daily feed intake also showed a time-dependent increase ($p < 0.001$; Table 2) over the 16 weeks rearing trial. In the first four weeks all genotypes excluding R11 had a similar daily feed intake. In the consecutive course, the brown genotypes showed a higher daily feed intake than the white ones until 16th week of age ($p < 0.05$), in which R11 achieved the significantly lowest daily feed intake of all four genotypes.

Feed to gain ratio of the genotypes also showed a time-dependent increase ($p < 0.001$; Table 2) over 16 weeks of rearing. In the first eight weeks only L68 and WLA achieved significantly lower feed to gain rations than R11. While no differences between genotypes occurred from the ninth to the twelfth week of age, brown genotypes showed lower feed to gain ratios than white ones in the last four weeks ($p < 0.001$). Cumulative feed to gain ratio of genotypes across the entire period did not differ.
Table 2. Growth performance of different genotypes from hatch to 16\textsuperscript{th} week of age (LSMeans; SEM; n = 140 (WLA), 76 (BLA), 147 (R11), 153 (L68)).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Body Weight (g/chick)</th>
<th>Daily Weight Gain (g/chick/d)</th>
<th>Daily Feed Intake (g/chick/d)</th>
<th>Feed to gain ratio (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hatch wk 4 8 12 16</td>
<td>1-4 5-8 9-12 13-16</td>
<td>1-4 5-8 9-12 13-16</td>
<td>1-4 5-8 9-12 13-16</td>
</tr>
<tr>
<td>WLA</td>
<td>38 209\textsuperscript{b} 538\textsuperscript{b} 919\textsuperscript{b} 1107\textsuperscript{c}</td>
<td>6.3\textsuperscript{b} 11.9\textsuperscript{b} 13.8\textsuperscript{b} 7.0\textsuperscript{b}</td>
<td>19.6\textsuperscript{b} 39.9\textsuperscript{b} 63.0\textsuperscript{b} 71.2\textsuperscript{b}</td>
<td>3.1\textsuperscript{ab} 3.4\textsuperscript{a} 4.6 10.2\textsuperscript{a} 5.2</td>
</tr>
<tr>
<td>BLA</td>
<td>38 224\textsuperscript{b} 535\textsuperscript{b} 923\textsuperscript{b} 1180\textsuperscript{b}</td>
<td>6.8\textsuperscript{b} 11.3\textsuperscript{b} 14.1\textsuperscript{ab} 9.4\textsuperscript{a}</td>
<td>21.5\textsuperscript{b} 43.4\textsuperscript{ab} 67.7\textsuperscript{a} 78.4\textsuperscript{a}</td>
<td>3.2\textsuperscript{ab} 3.8\textsuperscript{b} 4.8 8.3\textsuperscript{a} 5.1</td>
</tr>
<tr>
<td>R11</td>
<td>32 157\textsuperscript{c} 386\textsuperscript{c} 695\textsuperscript{c} 854\textsuperscript{d}</td>
<td>4.5\textsuperscript{c} 8.3\textsuperscript{c} 11.2\textsuperscript{c} 5.8\textsuperscript{c}</td>
<td>16.3\textsuperscript{b} 33.8\textsuperscript{d} 52.9\textsuperscript{c} 60.1\textsuperscript{c}</td>
<td>3.6\textsuperscript{c} 4.1\textsuperscript{c} 4.7 10.4\textsuperscript{a} 5.5</td>
</tr>
<tr>
<td>L68</td>
<td>34 251\textsuperscript{a} 607\textsuperscript{a} 1005\textsuperscript{a} 1249\textsuperscript{a}</td>
<td>7.9\textsuperscript{a} 12.9\textsuperscript{a} 14.5\textsuperscript{a} 8.9\textsuperscript{a}</td>
<td>20.2\textsuperscript{b} 46.1\textsuperscript{a} 70.5\textsuperscript{a} 77.4\textsuperscript{a}</td>
<td>2.6\textsuperscript{a} 3.6\textsuperscript{a} 4.9 8.7\textsuperscript{a} 4.9</td>
</tr>
<tr>
<td>SEM</td>
<td>5 5 5 6</td>
<td>0.2 0.2 0.2 0.2</td>
<td>1.3 1.3 1.4 1.5</td>
<td>0.2 0.2 0.3 0.4 0.4</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b,c,d}: LSMeans within columns with no common superscripts are significantly different (p < 0.05)

Table 3. Influence of genotype on the parameters of the Gompertz growth curve\textsuperscript{1} from hatch to 74\textsuperscript{th} week of age and the accuracy of data fit (LSMeans, SEM; n = 48 per genotype).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Estimated adult body weight (a) [g]</th>
<th>b</th>
<th>c</th>
<th>( t_{\text{max}} ) [weeks]</th>
<th>( R^2 )</th>
<th>RSD (g)</th>
<th>Estimated MDWG (g/chick/d)</th>
<th>Achieved average MDWG (g/chick/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WLA</td>
<td>1512\textsuperscript{b}</td>
<td>3.98\textsuperscript{b}</td>
<td>0.172\textsuperscript{a}</td>
<td>8.04\textsuperscript{c}</td>
<td>0.999</td>
<td>12</td>
<td>13.7\textsuperscript{ab}</td>
<td>14.5\textsuperscript{c}</td>
</tr>
<tr>
<td>BLA</td>
<td>1769\textsuperscript{b}</td>
<td>3.73\textsuperscript{b}</td>
<td>0.144\textsuperscript{c}</td>
<td>9.25\textsuperscript{a}</td>
<td>0.998</td>
<td>14</td>
<td>13.4\textsuperscript{b}</td>
<td>14.5\textsuperscript{a}</td>
</tr>
<tr>
<td>R11</td>
<td>1329\textsuperscript{c}</td>
<td>3.68\textsuperscript{b}</td>
<td>0.138\textsuperscript{a}</td>
<td>9.54\textsuperscript{a}</td>
<td>0.997</td>
<td>10</td>
<td>9.6\textsuperscript{c}</td>
<td>11.3\textsuperscript{b}</td>
</tr>
<tr>
<td>L68</td>
<td>1825\textsuperscript{a}</td>
<td>3.62\textsuperscript{b}</td>
<td>0.149\textsuperscript{a}</td>
<td>8.69\textsuperscript{b}</td>
<td>0.998</td>
<td>15</td>
<td>14.3\textsuperscript{a}</td>
<td>15.2\textsuperscript{a}</td>
</tr>
<tr>
<td>SEM</td>
<td>24</td>
<td>0.042</td>
<td>0.003</td>
<td>0.13</td>
<td>0.3</td>
<td></td>
<td>0.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

\textsuperscript{1} \( y(t) = a \cdot e^{-b \cdot e^{-ct}} \) with \( y = \) body weight at time \( t \) and the regression coefficients \( a \) (asymptotic limit = adult body weight), \( b \) and \( c \)

\( t_{\text{max}} \) is equivalent to the point of inflection (time of maximum weight gain); \( R^2 \) = coefficient of determination; RSD = residual standard deviation; SEM = standard error of mean; MDWG = maximum daily weight gain; \( a,b,c \): LSMeans within columns with no common superscripts are significantly different (p < 0.05)
Figure 2. Graphic presentation of the non-linear regression of growth data from hatch to the 74th week of age of 48 purebred laying hens of each genotype fitted to the Gompertz equation (a) and the derived course of daily weight gain (b) with emphasis of the genotype specific $t_{\text{max}}$ and the regression coefficients $a$ (asymptotic limit = adult body weight), $b$ and $c$.

Adaptation of growth data to the Gompertz function

Non-linear regression of growth data fitted to the Gompertz function (GOMPertz 1825) is summarized in Table 3. Genotype affected the equation parameters ($a$, $b$ and $c$) as well as $t_{\text{max}}$ and its associated maximum daily weight gain high significantly ($p < 0.001$). $t_{\text{max}}$ was achieved at 8.04 to 9.54 weeks of age. WLA reached maximum daily weight gain after 8.04 weeks firstly ($p < 0.05$). In contrast, BLA (9.25 weeks) and R11 (9.54 weeks) showed the
slowest growth rates ($p < 0.05$). R11 achieved the lowest and L68 the highest maximum daily weight gains at their specific $t_{max}$ ($p < 0.001$), respectively. Growth curves and their derivative, identical to the course of daily weight gain, are presented in Figure 2a and 2b. The asymptotic limit of the curves, which is equal to the estimated adult body weight, demonstrated highly significant ($p < 0.001$) differences between brown and white genotypes, while L68 (1825 g) and BLA (1769 g) showed no statistical differences. In contrast, the average adult body weight of white genotypes was calculated to be 1512 g (WLA) and 1329 g (R11) which differed significantly from each other ($p < 0.001$; Table 3). The course of daily weight gain showed a strong increase until the genotype-specific calculated $t_{max}$ and strongly decreased in the further course of the rearing trial. After the 40$^{th}$ week of age, daily weight gain curves of the genotypes approached the ordinate axis asymptotically.

Growing, feed intake and laying performance

Growth development and laying performance of the genotypes from the 23$^{rd}$ to the 74$^{th}$ week of age are summarized in Table 4, divided into four periods of 13 weeks each, and were significantly affected by genotype, age and their interaction ($p < 0.001$). To obtain a better overview, each performance trait is shown over time in Figure 3a-3e.

Body weight of the hens increased significantly with age ($p < 0.001$). During the whole trial, both brown layer lines were significantly heavier than both white layer lines (Table 4, Figure 2). Within the brown genotypes L68 and within the white genotypes WLA weighed more than their counterparts. From week 23 to 74 brown genotypes gained more than 80 g body weight. In low performing R11 body weight even increased by more than 110 g/hen. Only the high performing WLA did not alter body weight over the entire period.

During 13 laying months, daily feed intake (Table 4, Figure 3a) of the hens was nearly constant, but significant differences were observed between all four genotypes ($p < 0.05$). The high performing ones had a significantly higher daily feed intake compared to the low performing genotypes. Highest daily feed intake of 115 g/hen/d was recorded for BLA during week 23 to 35. Thereafter, daily feed intake of BLA decreased slightly up to the end of the trial, and did not differ from WLA feed intake at the end of the trial. During the entire trial WLA ingested approximately 100 g/hen/d constantly. The low performing genotypes differed
significantly from each other with a constant daily feed intake of approximately 75 g/hen/d (R11) and 92 g/hen/d (L68).

Laying maturity, defined as age at the first egg laid, was firstly reached by the hens of the high performing genotypes (Figure 3f). In comparison to the low performing genotypes, the high performing lines reached laying maturity four to five weeks earlier, in the 20th week of age ($p < 0.05$). On reaching maturity age, all genotypes but BLA (84%) weighed 90% of their adult body weight (Figure 3f).

Moreover, in the 364 days of the laying trial the high performing genotypes achieved an average number of 310 (BLA) to 325 (WLA) eggs, while the low performing genotypes reached 200 (R11) to 205 (L68) eggs. On average, 1.4 ± 0.5% (WLA), 2.7 ± 0.5% (BLA), 1.6 ± 0.5% (R11) and 0.8 ± 0.5% (L68) of the eggs showed defects such as shell-less, cracked-broken and double yolk eggs ($p = 0.0523$).

Due to the different age at laying maturity, egg production differed significantly at the beginning of the performance trial. In the first laying month high performing genotypes reached an egg production of more than 85 to 90% (Figure 3d), whereas the low performing hens showed an egg production of approximately 20% (R11) and 37% (L68), respectively, at the same time. All but R11 genotypes reached their maximum egg production in the second laying month. Hens of R11 reached their maximum egg production one month later. The maximum egg production of the high performing genotypes ranged from 93% (BLA) to 96% (WLA). Both low performing genotypes showed a lower egg production ($p < 0.05$) of maximal 67% (R11) and 74% (L68). In the following laying months egg production slightly decreased and persisted at approximately 75% in the high performing genotypes and 41 to 44% in the low performing genotypes until the end of the experiment. Results in Table 4 showed that the laying intensity of the high performing genotypes already started to decrease slightly after week 23 to 35, while the laying intensity of low performing genotypes increased until week 36 to 48 and decreased considerably thereafter.

Furthermore, the weight per egg (Figure 3c) ranged from approximately 40 g (R11, L68) to 50 g (WLA, BLA) at the beginning of the performance trial. In the following laying months egg weight slowly increased in all genotypes ($p < 0.001$). At the end of the trial, the eggs weight ranged from 54.0 g (R11) to 61.0 g (WLA, BLA, and L68).
Daily egg mass is illustrated in Figure 3b. In the first laying month the low performing genotypes began the trial with a significantly lower daily egg mass than the high performing ones. Maximum daily egg mass production was achieved by R11 in the third (31.4 g/hen/d), WLA (52.5 g/hen/d) and L68 (36.1 g/hen/d) in the fourth and BLA (53.6 g/hen/d) in the fifth laying month. To the end of the experiment, daily egg mass slowly decreased to 44.4 to 45.3 g/hen/d in the high performing genotypes and to 22.2 to 27.4 g/hen/d in the low performing layers. During the entire trial, high performing genotypes showed a higher daily egg mass production than the low performing ones ($p < 0.05$) while no differences were found neither between WLA and BLA nor between R11 and L68.

Due to the different age at laying maturity, feed to egg mass ratio (Figure 3e) of the low performing genotypes (L68: 5.68; R11: 8.92) differed significantly from the one of the high performing hens (WLA: 2.19; BLA: 2.58) in the first laying month (Figure 3e). From the second laying month, feed to egg mass ratio showed a nearly constant course. The four genotypes achieved their lowest level at the fourth to sixth laying month. In particular, the high performing WLA reached a feed to egg mass ratio of less than 1.95. The high performing BLA achieved its lowest feed to egg mass ratio of 2.02. Low performing genotypes showed significantly higher feed to egg mass ratios of more than 2.55. At the end of the experiment the feed to egg mass ratio of all genotypes slightly increased. The high performing hens (2.30) differed significantly from the low performing hens (R11: 2.85, L68: 3.20).

During the entire trial an average mortality of 8.9 % was recorded. The losses for each individual genotype are listed in the following ascending order: BLA 4.3 % - R11 8.3 % - L68 10.4 % and WLA 12.5 %.
### Table 4: Growth and laying performance of different genotypes from 23rd to 74th week of age subdivided in four periods of 13 weeks each (I: week 23 – 35; II: week 36 – 48; III: week 49 – 61; IV: week 62 – 74) (LSMeans, SEM; n = 48 per genotype).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Body weight (g/chick)</th>
<th>Feed intake (g/hen/d)</th>
<th>Laying intensity (%)</th>
<th>Egg weight (g/egg)</th>
<th>Egg mass (g/hen/d)</th>
<th>Feed to egg mass ratio (kg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I II III IV</td>
<td>I II III IV</td>
<td>I II III IV</td>
<td>I II III IV</td>
<td>I II III IV</td>
<td>I II III IV</td>
</tr>
<tr>
<td>WLA</td>
<td>1477 1492 1484 1480</td>
<td>104 101 102 106</td>
<td>94.3 94.1 87.9 83.1</td>
<td>53.0 55.4 56.3 59.1</td>
<td>50.3 52.2 49.5 49.1</td>
<td>2.07 1.93 2.06 2.16</td>
</tr>
<tr>
<td>BLA</td>
<td>1678 1755 1741 1761</td>
<td>115 112 109 108</td>
<td>90.2 89.9 84.8 78.0</td>
<td>54.6 59.2 59.3 60.2</td>
<td>49.3 53.2 50.3 47.0</td>
<td>2.33 2.11 2.17 2.30</td>
</tr>
<tr>
<td>R11</td>
<td>1226 1280 1302 1336</td>
<td>75 76 76 75</td>
<td>51.3 61.2 53.4 43.8</td>
<td>43.6 49.6 52.3 54.0</td>
<td>22.4 30.4 27.9 23.7</td>
<td>3.35 2.50 2.72 3.16</td>
</tr>
<tr>
<td>L68</td>
<td>1748 1787 1770 1829</td>
<td>92 93 92 94</td>
<td>60.7 65.4 53.6 46.8</td>
<td>46.5 53.9 58.0 60.5</td>
<td>28.2 35.2 31.1 28.3</td>
<td>3.26 2.64 2.96 3.32</td>
</tr>
<tr>
<td>SEM</td>
<td>25 25 25 25</td>
<td>2 2 2 2</td>
<td>3.0 3.0 3.0 3.0</td>
<td>0.5 0.5 0.5 0.5</td>
<td>1.6 1.6 1.6 1.6</td>
<td>0.25 0.25 0.25 0.25</td>
</tr>
</tbody>
</table>

a,b,c,d: LSMeans within columns with no common superscripts are significantly different ($p < 0.05$)

### Table 5. Effect of genotype on egg quality in the 40th, 65th and 74th week of age.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Egg weight (g/egg)</th>
<th>Shell (% of egg)</th>
<th>Yolk (% of egg)</th>
<th>Albumen (% of egg)</th>
<th>Yolk to albumen ratio</th>
<th>Yolk colour (Roche fan)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wk wk wk</td>
<td>wk wk wk</td>
<td>wk wk wk</td>
<td>wk wk wk</td>
<td>wk wk wk</td>
<td>wk wk wk</td>
</tr>
<tr>
<td>WLA</td>
<td>55.3b 57.1b 58.9a</td>
<td>13.6a 11.8ab 11.2b</td>
<td>29.3c 29.4c 29.8c</td>
<td>57.1b 58.8c 59.0b</td>
<td>0.51c 0.50c 0.50c</td>
<td>12.7b 13.5 13.1a</td>
</tr>
<tr>
<td>BLA</td>
<td>59.6a 60.0a 59.1a</td>
<td>12.9b 11.6b 10.3c</td>
<td>27.1c 26.8c 27.3c</td>
<td>60.0a 61.6a 62.4a</td>
<td>0.45d 0.43d 0.43d</td>
<td>12.4c 13.6a 12.9a</td>
</tr>
<tr>
<td>R11</td>
<td>49.3c 53.9c 53.2b</td>
<td>12.5b 12.4b 10.8bc</td>
<td>30.3c 32.3c 31.8c</td>
<td>57.2 55.3c 57.4e</td>
<td>0.53b 0.58b 0.55b</td>
<td>12.3c 12.4c 12.6b</td>
</tr>
<tr>
<td>L68</td>
<td>54.2b 59.7a 59.6a</td>
<td>11.9c 10.8c 11.8a</td>
<td>31.7b 33.5b 32.6b</td>
<td>56.4a 55.7c 55.6c</td>
<td>0.56b 0.61a 0.60b</td>
<td>13.0a 13.5a 12.9a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.6 0.6 0.7</td>
<td>0.1 0.1 0.1</td>
<td>0.3 0.3 0.3</td>
<td>0.3 0.3 0.3</td>
<td>0.01 0.01 0.01</td>
<td>0.1 0.1 0.1</td>
</tr>
</tbody>
</table>

1 Each value represents the least square mean of the laid eggs of 48 hens on three consecutive days each of three replicates

a,b,c,d: LSMeans within columns with no common superscripts are significantly different ($p < 0.05$)
Figure 3. Time-dependent course of performance parameters over 13 laying months (a-e) as well as age and body weight at laying maturity (f) of the four genotypes (LSMeans ± SE; n = 48 of each genotype).

Zeitabhängiger Verlauf der Leistungsparameter über 13 Legemonate (a-e) sowie Alter und Lebendmasse bei Legereife (f) der vier Genotypen.
Egg quality at the 40th, 65th and 74th week of age

Total egg weight, proportions of shell, yolk and albumen, the yolk color and the calculated yolk to albumen ratio (Table 5) were significantly influenced by genotype, age and genotype x age ($p < 0.001$).

In the 40th week of age, BLA eggs (59.6 g/egg) were significantly heavier eggs than those of other genotypes (WLA 55.3 g/egg; L68 54.2 g/egg; R11 49.3 g/egg). At the second and third examination date, eggs of all genotypes had increased their total egg weight, as age affected the absolute egg weight highly significantly ($p < 0.001$). At week 74, only R11 eggs (53.2 g/egg) differed from those of other genotypes (58.9 to 59.6 g/egg; $p < 0.05$).

In contrast to the absolute egg weight, WLA (13.6 %) showed a significantly higher relative proportion of egg shell in week 40 compared to the other genotypes (BLA 12.9 %; R11 12.5 %; L68: 11.9 %). From week 40 to 74 the relative proportion of egg shell decreased significantly in WLA, BLA and R11 (about 1.7 to 2.6 % of total egg weight; $p < 0.001$), while the egg shell of L68 remained constant at 11.8 % at last examination date.

Eggs of high performing genotypes (27.1 to 29.8 %) showed significantly lower proportions of yolk than the eggs of the low performing ones (30.3 to 33.5 %) at all three examination times. Egg yolk proportion of high performing genotypes remained constant during the trial, while the eggs of low performing genotypes showed a slight increase in yolk proportion ($p < 0.001$).

A reverse situation was found in the albumen proportion. Eggs of WLA and BLA (57.1 to 62.4 %) had a significantly higher albumen proportion than those of R11 and L68 (55.3 to 57.4 %). The albumen proportion of the high performing genotypes increased by time ($p < 0.001$), while eggs of R11 and L68 remained largely constant in this proportion at the three examination times.

Purebred layers of the low performing genotypes showed a significantly higher yolk to albumen ratio than the high performing genotypes. The yolk to albumen ratio of the low performing hens increased by time, while that of the high performing hens remained constant across the three measurement dates ($p < 0.001$).

Finally, L68 eggs showed the most intensive yolk color with 13.0 on the Roche scale at the first examination time ($p < 0.05$). In course of the examination the time affected the yolk color ($p < 0.001$), as the eggs of WLA, BLA and L68 achieved their most intensive coloring at
week 65 (13.4 to 13.5), while the R11 yolks (12.6) had their most intensive color at week 74. From the second to the third time of examination, yolk color of WLA, BLA and L68 declined again by 0.5 points on average to approximately 13.0 on the Roche scale.

Discussion
The objective of the present study was to examine the influence of divergent genotypes on performance related parameters. The experimental design allows to assess the effect of both components separately, performance divergence and phylogenetic divergence. Phylogenetic relationship between white layers and brown layers were described previously (GRANEVITZE et al. 2009; LYIMO et al. 2014).

Significant differences were found in the four genotypes studied concerning several performance parameters. In accordance with PREISINGER (2000), results of this study confirmed that brown layer genotypes had a higher body weight than white layers. Higher body weight went along with a significantly higher feed intake in brown genotypes in contrast to their white counterparts, resulting from an absolutely higher maintenance requirement of brown layers (PREISINGER 2000).

Because of identical conditions in feeding and housing, differences in daily feed intake and laying performance are likely to result from the genetically determined performance potential of the studied genotypes. Besides the significant differences between high and low performing genotypes in several laying performance traits, also age at onset of laying was significantly different between the high and low performing genotypes. Laying maturity is an important trait which is affected by selection and has a great importance to the life output of laying hens (POGGENPOEL and DUCKITT 1988). WLA and BLA in this trial reached laying maturity four to five weeks earlier than the low performing genotypes. As described by POGGENPOEL and DUCKITT (1988) the intensive selection on egg production was closely connected with the intensive selection on sexual maturity. This fits well to the findings of HORN and SÜTÖ (2000) that today’s layers start to lay about 15 to 20 days earlier than 20 years ago.

Furthermore, these authors reported that the body weight of white layers remain constant, while the egg weight and the total egg mass increased. In case of body weight, the high performing brown layers (BLA) showed a significantly lower body weight than the low
performing ones after four weeks of rearing. This significant difference remained until the end of the laying performance trial in the 74th week of age. Within the high performance level the white layers (WLA) showed a significantly lower body weight than BLA. This circumstance is also well depicted by the calculated growth curves in our study. The Gompertz equation is frequently used in poultry (GOUS et al. 1999; SAKOMURA et al. 2005). Body weight data of all four genotypes were adapted to the Gompertz equation and its derivative, and showed a good fit to the chosen model with $R^2$ ranging from 0.997 (R11) to 0.999 (WLA). As a result, this growth equation (Figure 2, Table 3) could be useful for further experiments under changing environmental conditions to determine the efficiency of nutrient utilization, or to predict daily energy, protein and mineral requirements (DARMANI KUHI et al. 2010) of the genotypes studied. Significant differences were observed among the genotypes for several curve parameters, especially the age at maximum daily weight gain ($t_{\text{max}}$). In contrast to BLA, WLA achieved the maximum weight gain first and reached, as well as R11 and L68, 90% of its adult body weight at age of laying maturity. These findings emphasize the fast development of high performing White Leghorn layers regarding their body weight. Intensive selection on early sexual maturity reduced the age at first egg (POGGENPOEL and DUCKITT 1988) and age at reaching the asymptotic body weight (SZYDLOWSKI and SZWACZKOWSKI 2001).

In addition to the laying performance, egg quality was analyzed. It shows that genotype, age and their interaction had a highly significant influence on the evaluated parameters. According to the findings of several other authors (HEIL and HARTMANN 1997; LEDVINKA et al. 2000; VITS et al. 2005), in week 40 and week 65 the examined eggs of brown hens were heavier than those of white hens, while at week 74, differences between BLA, L68 and WLA were no longer statistically detectable. In agreement with RIZZI and CHIERICATO (2005) and JOHNSTON and GOUS (2007), the absolute egg weight increased with the age of the hens in the studied genotypes. Concerning the effect of age on egg proportions, there are contradicting findings reported in the literature (ROSSI and POMPEI 1995; SUK and PARK 2001; SILVERSIDES and SCOTT 2001; YANNAKOPOULOS et al. 1994). In our study, eggs of high performing hens showed a decreasing proportion of shell and yolk with age, but increasing proportion of albumen. In comparison, eggs of low
performing hens decreased in proportion of egg shell, but increased for egg yolk, and remained constant in albumen content.

Besides the time-dependent effect and the differences between high and low performing genotypes on egg quality, several authors made statements about the effects of phylogeny. LEYENDECKER et al. (2001) found significantly higher yolk proportions in white eggs than in those of brown layers. Our results indicate that the eggs of high performing brown layers had lower yolk proportions than those of the high performing white layers. In case of the low performing genotypes, however, an opposite relation was found between white and brown layers. Furthermore, the white hens of present study showed a significantly higher egg shell proportion than brown hens. The findings of LEDVINKA et al. (2000) contradict our results, while BASMACIOGLU and ERGUL (2005) found no significant effect of genotype on shell percentage by comparing Babcock-300 (white layers) with Isa-Brown (brown layers). This suggested that general statements about egg quality parameter without consideration of the studied genotype should not be made, as genetic influence on egg quality parameters is very strong (BUSS and GUYER 1982; STEINHILBER 2005; FLOCK et al. 2007).

In addition to egg quality, proportion of defect eggs was recorded. In contrast to WOLC et al. (2012), the present study did not confirm the assumption that high producing hens had a lower frequency of egg defects in general. The lowest proportion of defect eggs was observed in low performing brown hens. While there was hardly any difference between white hens of WLA (1.4 %) and R11 (1.6 %), proportion of defect eggs differed markedly between brown hens of L68 (0.8 %) and BLA (2.7 %).

Genotypes of same performance level and with a distant phylogenetic relationship (WLA/BLA and R11/L68) showed similarities in several traits with differences less than five percentage. Other traits differed significantly between five and ten percentage in high performing genotypes (e.g. egg yolk proportion, daily feed intake and feed to egg mass ratio) and between 20 and 40 percentage in low performing genotypes (e.g. body weight, daily feed intake, egg weight and daily egg mass). Considering the phylogenetic relationship (WLA/R11 and BLA/L68), differences of about 30 to 100 percentage could be found in performance related traits. However, differences in egg quality parameters were much lower (four to nine percentage) between closely related strains.
Conclusions
The results of this study fitted well with the intended performance divergence between high and low performing genotypes in the established experimental design (Fig.1). Firstly, recorded data of daily feed intake, growth and laying performance under conditions of identical feeding and housing gave an initial impression of the performance potential of the studied genotypes. Secondly, such data could be used to calculate performance-dependent nutrient requirements of the four genotypes in further experiments. In those studies the genotypes should be stressed by changing environmental conditions (e.g. via nutrition, infectious diseases, challenging housing conditions) and their physiological reactions could be studied concerning the adaptation ability to such new conditions.

Acknowledgements
The authors gratefully acknowledge Silvia Wittig, Ines Weinholz and Gabi Orlowski for their participation at the care of the experimental animals, the sample and data collection and the practical realization of the experiments. Furthermore, the authors acknowledge Annerose Junghans for her participation at the sample preparation.

Zusammenfassung
Wachstumsentwicklung in den ersten 16 Lebenswochen in einem Aufzuchtversuch, einer sechswöchigen Vorlegephase und einem angeschlossenen Leistungsversuch über 13 Legemonaten (23. – 74. Lebenswoche) charakterisiert. Die untersuchten Leistungsmerkmale wurden durch den Genotyp, das Alter sowie deren Interaktion höchst signifikant beeinflusst (p ≤ 0.001). Als Folge der Selektion auf hohe Legeleistung zeigten selektierte Linien eine signifikant höhere Leistung als Nichtselektierte. Die hochleistenden Genotypen hatten eine Legeintensität von durchschnittlich 85 bis 90 %, eine tägliche Eimasseproduktion von ca. 50 g/Henne/d sowie eine Futterverwertung von 2.1 bis 2.3 kg/kg. Die minderleistenden Genotypen hatten hingegen eine Legeintensität von durchschnittlich 52 bis 56 %, eine tägliche Eimasseproduktion von 26 bis 31 g/Henne/d sowie eine Futterverwertung von ca. 3.0 kg/kg. Hinsichtlich des durchschnittlichen Eigewichtes unterschied sich lediglich R11 mit 50 g/Ei von den übrigen Versuchslinien mit 55 bis 58 g/Ei. Unabhängig von ihrer Leistung zeigten braune Hennen über den gesamten Versuch eine signifikant höhere Lebendmasse als weiße Hennen.

Untersuchungen der Eiqualität zeigten, dass hochleistende Linien einen signifikant höheren Eiklaranteil (57.1 bis 62.4 %), gleichzeitig aber einen signifikant niedrigeren Eidotteranteil (26.8 bis 29.8 %) aufwiesen als minderleistende Linien (Eiklar: 55.3 bis 57.4 %, Dotter: 30.3 bis 33.5 %). Weiße Hennen (10.8 bis 13.6 %) besaßen einen signifikant höheren Schalenanteil als braune Linien (10.3 bis 12.9 %).


Stichworte: Versuchsdesign, Küken, Junghennen, Legehennen, Genotypen, Wachstum, Legeleistung, Eiqualität, Gompertz-Funktion
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Effects of Long-term Graded L-arginine Supply on Growth Development, Egg Laying and Egg Quality in Four Genetically Diverse Purebred Layer Lines

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\textbf{Journal of Poultry Science (JPS)}

Volume 53

DOI: 10.2141/jpsa.0150067

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Abstract
The present study was conducted to examine effects of long-term graded L-arginine supply on growth development, egg laying and egg quality in four genetically diverse purebred layer lines. The study comprised a rearing trial from hatch to week 16 and a following laying performance trial from week 17 to 41. After hatch 150 one-day-old female chicks of each genotype were distributed to three diets. The experimental diets were equivalent to 70, 100 and 200 % L-arginine of age-specific recommended level (NRC 1994) and offered ad libitum to chicks (hatch to week 7), pullets (week 8 to 16) and hens (week 17 to 41). However, hens’ diets were quite low in crude protein. After a pre-laying period from week 17 to 21 thirty-six pullets of each group were used further in the laying performance trial. Independent of chicken’s genetic background, insufficient L-arginine supply caused lower body weight, daily weight gain and daily feed intake during the entire experiment ($p < 0.001$) and induced lower laying intensity and daily egg mass production in the laying period ($p < 0.05$). Parameters fitted to Gompertz function suggested higher adult body weight in L-arginine supplemented birds compared to insufficient supplied ones ($p < 0.01$). Groups fed with insufficient L-arginine reached age of maximum daily weight gain later and showed lowest maximum daily weight gain ($p < 0.001$). As a consequence of limitations in dietary L-arginine and crude protein, high performing genotypes decreased strongly in body weight, daily feed intake and performance compared to the low performing genotypes. In conclusion, L-arginine modified the amount of weight gain and feed intake, especially in growing chicks and pullets independent of genetic background. The high performing hens were more nutritionally stressed than the low performing ones, because concentrations of dietary crude protein were relatively low.

**Key words:** L-arginine, egg quality, genotypes, Gompertz equation, growth, laying performance

**Introduction**
L-arginine (Arg), an essential amino acid in poultry, plays a decisive role in multiple physiological processes like growth and feathering, and serves as precursor of proteins, creatine, polyamines, L-proline, various hormones and nitric oxides (reviewed in: KHAJALI...
and WIDEMAN 2010). Nitric oxides serve multiple functions in immune system (KWAK et al. 2001; TAYADE et al. 2006), vasomotory regulation (LORENZONI and RUIZ-FERIA 2006; TAN et al. 2006) and nervous system (GASKIN et al. 2003; FARR et al. 2005). Due to avian uricotelism and its underlying functionally incomplete urea cycle (TAMIR and RATNER 1963), chickens are unable to synthesize Arg de novo, highly depend on dietary Arg, and have an absolute Arg requirement. KWAK et al. (1999, 2001) describe that metabolisable plasma Arg is directly influenced by dietary Arg. The required magnitude depends on a large variety of environmental factors like bird’s age and feather coverage (BEQUETTE 2003), source of dietary protein (BURTON and WALDROUP 1979; CUCA and JENSEN 1990), imbalances between dietary amino acids (KESHAVARZ and FULLER 1971 a; CHAMRUSPOLLERT et al. 2004, JAHANIAN 2009) as well as ambient temperatures and stressful conditions (BRAKE et al. 1998; SRINONGKOTE et al. 2004).

Apart from environmental factors, nutrient utilization and metabolism are also affected by genetic influences (SIMOPOULOS 2002). As feather protein contains high amounts of Arg and glycine (BLOCK 1939), HEGSTEDT et al. (1941) find different Arg requirements between White Leghorn and Barred Plymouth Rock chicks in the first weeks of age due to their diverse rapidity in feathering. White Leghorns show further a diverse Arg utilization and requirement for physiological functions due to genetic divergence in lysine metabolism (Lys; NESHEIM and HUTT 1962; HUTT and NESHEIM 1966).

Several authors associate selection with undesirable side-effects such as deficiencies in physiological, immunological and reproduction traits (MILLER et al. 1992; LIU et al. 1995; RAUW et al. 1998), and consider selection for high production efficiency as process that exacerbates genetically determined differences in nutrient requirements between genotypes (GTs). Due to genetic adaptation, metabolic resources have to be optimally distributed between maintenance to cope with the environment in which animals are kept, and production traits (BEILHARZ et al. 1993). Because selection aims at minimizing metabolic resources not needed for maintenance, VAN DER WAAIJ (2004) and MIRKENA et al. (2010) hypothesized that high performing genotypes (HPGTs) have a reduced capacity to compensate unexpected environmental changes like nutritional limitations and imbalances compared to low performing genotypes (LPGTs).
Adapting this hypothesis we conducted a long-term study from hatch to the end of 41\textsuperscript{st} week of age with four GTs of purebred layer lines differing in phylogenetic origin and performance. Different grades of dietary Arg served as nutritional-environmental stress factor during the entire trial. The objective was to examine long-term effects of dietary Arg interacting with chickens’ GTs on growth development and performance.

**Materials and Methods**

*Experimental design and diets*

The used animal model has been described by LIEBOLDT et al. (2015) previously. Four purebred layer lines differing in performance and phylogenetic origin were examined. Two commercial HPGTs (WLA and BLA) taken from breeding programme of Lohmann Tierzucht GmbH were contrasted to two LPGTs (R11 and L68). The latter ones were maintained as non-selected resource populations at the Institute of Farm Animal Genetics in Mariensee. Both white layer lines (WLA and R11) were of White Leghorn origin and phylogenetically closely related, but distant from the Rhode Island Red higher performing BLA and its low performing counterpart L68 (New Hampshire).

Purified diets were not appropriate to guarantee animal welfare and practicability because the present study lasted for several months. Due to its low Arg content corn gluten meal served as main protein source in the experimental diets (Table 1). According to the recommendations of the National Research Council (NRC 1994) three experimental diets were calculated for the age-groups chicks and growers (hatch to week 7), growers and pullets (week 8 to 16) and laying hens (week 17 to 41) each. The diets of each age-group comprised a basal diet with no Arg supplementation (low Arg, LA), a low Arg (adequate Arg, AA) and a high Arg supplemented diet (high Arg, HA). The basal diet was supplemented with any deficient essential amino acid other than Arg. For AA and HA, L-arginine (free base, crystalline, 99 %, Europepta, Hannover, Germany) was added to the basal diet in place of corn. The diets were equivalent to 70, 100 and 200 % Arg of the recommended level (NRC 1994), respectively. With reference to immunonutritional research dealing with dietary Arg (KWAK et. 1999, 2001; TAYADE et al. 2006; TAN et al. 2014), we regarded these graded dietary Arg concentrations as well-suited for further immunological research in the reared chickens.
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).

Rearing trial
After hatch 150 female one-day-old chicks of each GT were equipped with individual wing-tags, vaccinated against MD and ND, and distributed to the three diets (Table 1) for chicks and growers for the first seven weeks (12 experimental groups). Afterwards groups were fed with corresponding diets for growers and pullets from week 8 to 16. Light was provided for 24 hours on day 1 and 2. From day 3 onwards light was reduced to 15 hours daily in the first week of age. From week 1 to 7 daily light period was shortened stepwise by one hour a week to 9 hours and maintained until the end of rearing. Temperature programme followed usual specifications of chickens reared for laying. Chicks of each group (n = 50) were housed in five floor-range pens of ten chicks each, equipped with nipple drinkers and feeding trough. Feed and water were provided ad libitum during the entire trial. Chicks were weighed once a week from hatch to week 4 and body weight (BW) was recorded every second week from week 6 to 16. Residual feed was recorded weekly. Daily weight gain (DWG), daily feed intake (DFI), and feed conversion ratio (FCR) were calculated on basis of recorded data.

Performance trial of laying hens
At the end of rearing, thirty-six 17-week-old pullets of each experimental group were moved to a layer facility. Each group was allocated to one pen of a floor-range system equipped with a feeding trough, nipple drinkers, perches, scratch area, deep pit and nests. According to the rearing trial, corresponding hen diets with graded Arg were fed further to hens of each group from week 17 onwards (Table 1). Feed and water were provided ad libitum. From 17th week of age onwards daily light duration was increased by half an hour per week to 14 hours of light at 23rd week of age. After a pre-laying period from week 17 to 21 with continuous data recording, the performance trial comprised five 28-day laying periods and lasted from week 22 to 41.
Laying hens were weighed at the end of each 28-day laying period. The number of laid eggs was recorded daily. For each laying period egg weight (EW) was monitored by collecting all laid eggs of each pen on four consecutive days in a two-week interval. Residual feed was recorded weekly. Based on recorded data daily feed intake (DFI), laying intensity (LI), daily egg mass (DEM) and the feed to egg mass ratio (FEM) were calculated.

**Egg quality parameters**

At the end of each laying period (25th, 29th, 33rd, 37th and 41th week of age) all laid eggs were collected on four consecutive days (25th week: 665 eggs, 29th week: 790 eggs, 33rd week: 640 eggs, 39th week: 580 eggs and 41st week: 430 eggs). Eggs were weighed and egg yolk and albumen were separated. Weight of shell, including inner shell membrane, and weight of yolk were recorded. Weight of albumen was determined by subtracting yolk and shell weight from the original egg weight. Egg components were presented in percentage as proportions of the total egg weight. Yolk colour (YC) was estimated by using a Roche-fan (15 fans, F. Hoffmann-La Roche Ltd., Basel, Switzerland).

As protein and amino acid deposition in eggs were considered to be genetically determined and virtually insensitive to dietary manipulations (ROLAND 1980 a, b; LEESON 1993; HUSSEIN and HARMS 1994), we assume that the Arg concentration in egg yolk (11.36 mg Arg/g) and egg albumen (5.92 mg Arg/g) described by BERGQUIST (1979) were applicable to our examined eggs. Consequently, daily Arg transfer into total egg and its components yolk and albumen were calculated by multiplying daily egg mass by the proportion of egg yolk or albumen and the corresponding Arg concentration described by BERGQUIST (1979). Dividing daily Arg transfer into egg by daily dietary Arg intake, the partial Arg utilization for egg production was determined.
Table 1. Composition, analysed and calculated nutrient contents of the experimental diets.

<table>
<thead>
<tr>
<th>Ingredients (g/kg diet)</th>
<th>Chicks and growers (week 1 - 7)</th>
<th>Growers and pullets (week 8 - 16)</th>
<th>Laying hens (week 17 - 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LA</td>
<td>AA</td>
<td>HA</td>
</tr>
<tr>
<td>Barley</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>Wheat</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Triticale</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corn</td>
<td>399.0</td>
<td>396.0</td>
<td>386.0</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>150.0</td>
<td>150.0</td>
<td>150.0</td>
</tr>
<tr>
<td>Lucerne pellets</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>39.8</td>
<td>39.8</td>
<td>39.8</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>33.3</td>
<td>33.3</td>
<td>33.3</td>
</tr>
<tr>
<td>Premix ^1</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Premix ^2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Premix ^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-lysine HCl</td>
<td>4.9</td>
<td>4.9</td>
<td>4.9</td>
</tr>
<tr>
<td>L-arginine</td>
<td>-</td>
<td>3.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-threonine</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-valine</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-isoleucine</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Chemical composition, g/kg diet

<table>
<thead>
<tr>
<th></th>
<th>Chicks and growers (week 1 - 7)</th>
<th>Growers and pullets (week 8 - 16)</th>
<th>Laying hens (week 17 - 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter ^4</td>
<td>897.6</td>
<td>893.0</td>
<td>896.6</td>
</tr>
<tr>
<td>Crude ash ^4</td>
<td>60.0</td>
<td>57.2</td>
<td>58.2</td>
</tr>
<tr>
<td>Crude protein ^4</td>
<td>174.1</td>
<td>176.0</td>
<td>186.9</td>
</tr>
<tr>
<td>Kjeldahl Nitrogen ^a</td>
<td>27.9</td>
<td>28.5</td>
<td>32.0</td>
</tr>
<tr>
<td>Crude fat ^5</td>
<td>40.1</td>
<td>38.6</td>
<td>37.2</td>
</tr>
<tr>
<td>Crude fiber ^5</td>
<td>33.6</td>
<td>31.0</td>
<td>34.2</td>
</tr>
<tr>
<td>Starch ^6</td>
<td>459.7</td>
<td>457.7</td>
<td>449.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20.6</td>
<td>20.1</td>
<td>20.3</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>9.8</td>
<td>10.0</td>
<td>10.2</td>
</tr>
<tr>
<td>Calcium ^7</td>
<td>12.7</td>
<td>12.6</td>
<td>13.1</td>
</tr>
<tr>
<td>AMEn (MJ/kg ^5)</td>
<td>12.0</td>
<td>12.0</td>
<td>12.1</td>
</tr>
<tr>
<td>Methionine ^6</td>
<td>3.34</td>
<td>3.34</td>
<td>3.32</td>
</tr>
<tr>
<td>Arginine ^8</td>
<td>6.54</td>
<td>9.53</td>
<td>19.50</td>
</tr>
</tbody>
</table>

^1 Premix – chicks: feed additives (per kg premix): Vitamin A, 1,200,000 IU; Vitamin D3, 350,000 IU; Vitamin E, 4,000 mg; Vitamin B1, 250 mg; Vitamin B2, 800 mg; Vitamin B6, 600 mg; Vitamin B12, 3.200 µg; Vitamin K3, 450 mg; Nicotinamide, 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 D3, 200,000 IU; Vitamin E, 2,500 mg; Vitamin B1, 250 mg; Vitamin B2, 500 mg; Vitamin B6, 400 mg; Vitamin B12, 1,850 µg; Vitamin K3, 300 mg; Nicotinamide, 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 Premix – hens: feed additives (per kg premix): Vitamin A, 1,000,000 IU; Vitamin D3, 250,000 IU; Vitamin E, 2,000 mg; Vitamin B1, 250 mg; Vitamin B2, 700 mg; Vitamin B6, 400 mg; Vitamin B12, 2,000 µg; Vitamin K3, 400 mg; Nicotinamide, 4,000 mg; Calcium-D-pantothenate, 1,000 mg; Folic acid, 60 mg; Biotin, 2,500 µg; Choline chloride, 40,000 mg; Fe, 4,000 mg; Cu, 1,000 mg; Mn, 10,000 mg; Zn, 8,000 mg; I, 120 mg; Se, 25 mg; Co, 20,5 mg; Butylated hydroxy toluene (BHT), 12,500 mg; Beta-carotene, 400 mg; Canthaxanthin, 400 mg
^4 Analyzed
^5 Apparent metabolizable energy concentrations corrected to zero nitrogen balance (AMEn), 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
Dry matter and crude nutrients of feed
Diets (Table 1) were analysed for dry matter, crude ash, crude fat, crude fibre, starch, sucrose, phosphorous, calcium and Kjeldahl N according to the methods of the Association of German Agricultural Analytic and Research Institutes (VDLUFA; BASSLER 1993). Dietary crude protein of the basal diets was calculated by multiplying Kjeldahl N by 6.25. As nitrogen content of Arg is twice as high as that of crude protein the nitrogen differences between Arg supplemented diets and the basal diet were multiplied by 3.13 in order to avoid an overestimation of dietary crude protein in the supplemented diets. The apparent metabolisable energy concentration corrected to zero nitrogen balance (AME_N) of diets was calculated according to the energy estimation equation of the World’s Poultry Science Association (VOGT 1986). In order to calculate the concentrations of amino acids in the experimental diets appropriately, amino acid containing feed components others than those supplemented in their free forms were analyzed for their containing amounts of amino acids by ion exchange chromatography according to the description of analytical methods in AMINODat® 4.0 (EVONIK INDUSTRIES 2010).

Modelling of growth curves
The growth function of GOMPertz (1825) was fitted regressively to time-dependent individually recorded growth data (cumulative growth; n = 36 per group) from hatch to the end of the 41st week of age using procedure “nonlinear regression” of the software package “Statistica 12.0 for the Windows™ Operating System” (STATSOFT INC. 2014). The equation parameters were estimated using the iterative Quasi-Newton method.

\[ y(t) = a \cdot e^{-bc^{-t}} \]

Where \( y(t) \) = body weight (g) of the hen at time \( t \), expressed as a function of \( a \); \( a \) = adult body weight (g) of the hen (asymptotic limit); \( b, c \) = parameters of the function (regression coefficients); and \( t \) = time (weeks). The age at maximum body weight gain (\( t_{\text{max}} \)) that is equivalent to the point of inflection of the cumulative, sigmoid growth curve was calculated by the second derivative of the cumulative growth function:

\[ t_{\text{max}} = \frac{\ln b}{c} \]

The maximum daily weight gain was computed by applying the group specific calculated \( t_{\text{max}} \) in the derivative of the cumulative growth function for the corresponding experimental group.
Statistical analyses

Statistical analysis of performance traits, egg quality parameters and the partial Arg utilization was carried out by means of a three factorial analysis of variance (ANOVA) with genotype, diet and age as well as their interactions as fixed effects. For traits measured repeatedly on the same animal a “repeated” statement was considered in the statistical model to account for similarities within subjects. For calculated growth function parameters, a two factorial ANOVA with genotype and diet as well as their interaction as fixed effects was carried out. In both cases the Tukey-Kramer test was applied for a multiple comparison of means. Data were reported as least square means and pooled standard errors. Differences were considered to be statistically significant for $p < 0.05$. The ANOVA were performed using the procedure MIXED of the software package SAS 9.4 (SAS INSTITUTE INC. 2012).

Results

Rearing trial

Growth development and rearing performance are presented in Table 2. BW was significantly affected by GT, diet and age as well as their two-factorial interactions. At hatch BW did not differ between GTs, but it increased time-dependently and GTs differed from week 4 onwards ($p < 0.001$). Brown GTs achieved a higher BW than white ones ($p < 0.001$), and L68 and WLA reached the highest BW within phenotypes. During the entire trial R11 showed the lowest BW, whereas HPGTs differed between each other in week 16 only. From week 4 onwards the insufficient Arg diet caused the lowest BW in all GTs, but the highest BW was recorded in chicks fed with AA and HA ($p < 0.001$), equally. GTs fed with LA and HA showed differences as described above ($p < 0.001$), but AA induced no differences between WLA and BLA. Differences between AA and HA occurred in L68 only, as AA caused higher BW ($p < 0.001$).

DWG was influenced by GT, diet and age as well as the interactions GT x age and diet x age ($p < 0.001$). From week 5 onwards DWG differed between GTs for the first time, and R11 gained the lowest and L68 the highest BW daily ($p < 0.001$). However, HPGTs gained BW to the same extent during the entire rearing period. From week 9 to 12 all GTs but R11 reached their highest DWG and they decreased afterwards ($p < 0.001$). R11 achieved its highest DWG in the last four weeks of rearing. At the end of trial L68 differed from the other GTs only. The Arg deficit group had the lowest DWG among all GTs ($p < 0.001$).
DFI was significantly affected by GT, diet and age as well as their two-factorial interactions. Although DFI increased age-dependently \((p < 0.001)\), HPGTs did not differ during the rearing. Independent of diet L68 had the highest and R11 the lowest DFI from week 5 onwards \((p < 0.001)\). In contrast to the deficit diet, AA caused higher DFI among all GTs from week 5 to 8 and in L68 from week 9 to 16 as well \((p < 0.01)\).

FCR was only affected by GT, age and their interaction \((p < 0.01)\). A dietary influence on FCR did not occur. The lowest FCR was performed during the first 4 weeks of rearing, in which HPGTs significantly differed from LPGTs. Afterwards FCR increased age-dependently \((p < 0.001)\), and GTs did not differ anymore. In general, the cumulative FCR of the entire rearing did not differ between GTs and diets.

During rearing an average mortality of 1.22 % was recorded. Although 90 % of losses occurred during the first five weeks of age, specific influences were not found.

**Adaptation of the Gompertz function to the growth data**

The Gompertz function was fitted to growth data by nonlinear regression. The results are summarized in Table 3 and graphically presented in Figures 1a) – b) and 1e) – f). The calculated parameters of the group-specific growth functions, \(t_{\text{max}}\), and its corresponding maximum DWG were significantly affected by GT and diet. The asymptotic limit of growth curves, equal to estimated adult BW, was lower in white GTs compared with brown ones \((p < 0.001)\). In addition, HA fed brown GTs showed higher asymptotic BW than those of the deficit group \((p < 0.01)\). On the other hand, AA fed WLA reached \(t_{\text{max}}\) at first, and LA fed BLA grew slowest \((p < 0.05)\). Independent of diet, \(t_{\text{max}}\) of WLA differed significantly from those of the other GTs that showed no difference between each other. However, AA caused fastest growth among all GTs and differed significantly from LA and HA, equally.

Figures 1c) – d) and 1g) – h) present the derivatives of growth curves, identical to the course of DWG. The graphs show a significant DWG increase until group-specific \(t_{\text{max}}\) with its corresponding estimated maximum DWG. Brown GTs achieved higher maximum DWG than white GTs \((p < 0.001)\). However, the deficit groups induced significantly lower maximum DWG at \(t_{\text{max}}\) among all GTs. Consequently, curves of deficit groups were flatter than those of AA and HA \((p < 0.001)\). After reaching maximum, DWG decreased strongly and approached nearly zero-gain asymptotically after the 40th week of age.
<table>
<thead>
<tr>
<th></th>
<th>WLA</th>
<th>BLA</th>
<th>R11</th>
<th>L68</th>
<th>PSEM</th>
<th>GT</th>
<th>DIET</th>
<th>AGE</th>
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<th>GTxAge</th>
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*PSEM: LSMMeans values with PSEM (n = 50 chicks/experimental group) in the same row with different superscripts are significantly different (p < 0.05)  
n.s. = not significant*
Figure 1. Graphic presentation of non-linear regression of genotypes’ growth data depending on L-arginine supply from hatch to week 41 fitted to the Gompertz equation \( y(t) = a \cdot e^{-b \cdot e^{-ct}} \) with \( y \) = body weight at time \( t \) and the regression coefficients \( a \) (asymptotic limit = adult body weight), \( b \) and \( c \). (a-b and e-f) and derived course of daily weight gain (c-d and g-h) with emphasis of the genotype specific \( t_{\text{max}} \) (n = 36).
Table 3. Effect of Arg supply and genotype on results of time-dependent individual growth data fitted to the Gompertz function¹.

<table>
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<tr>
<th></th>
<th>WLA</th>
<th>BLA</th>
<th>R11</th>
<th>L68</th>
<th>PSEM</th>
<th>GT</th>
<th>DIET</th>
<th>GTxDIET</th>
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<tr>
<td>a (g/chick)</td>
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<td>1756</td>
<td>1342</td>
<td>1887</td>
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<td>26</td>
<td>26</td>
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<td>12.3</td>
<td>12.3</td>
<td>13.4</td>
<td>0.2</td>
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<tr>
<td>Achieved MDWG (g/chick/d)</td>
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<td>12.9</td>
<td>12.9</td>
<td>14.6</td>
<td>0.3</td>
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</tr>
</tbody>
</table>

α-γ: LSMeans values with PSEM (n = 36 chicks/experimental group) in the same row with different superscripts are significantly different (p < 0.05)

¹: \( y(t) = ae^{-be^{-ct}} \) with \( y \) = body weight at time \( t \) and the regression coefficients \( a \) (asymptotic limit = adult body weight), \( b \) and \( c \)

\( t_{max} \): equivalent to the point of inflection (time of maximum weight gain); \( R^2 \): coefficient of determination; RSD = residual standard deviation; PSEM = pooled standard error of mean; MDWG = maximum daily weight gain; n.s. = not significant

\( GT \): genotype; \( DIET \): diet; \( GTxDIET \): genotype x diet interaction.
Growth, feed intake and laying performance

Table 4 summarizes growth development and laying performance of GTs from week 22 to 41. Hens’ BW was significantly affected by GT, diet and age as well as their interactions. Independent of diet and age, brown hens achieved higher BW than white ones ($p < 0.001$), and L68 and WLA showed highest BW within phenotypes. BW of GTs except L68 increased until week 25 ($p < 0.001$), whereas L68 gained BW until week 33 and remained constant afterwards like R11 ($p < 0.001$). On the other hand, HPGTs lost weight continuously and differed from each other during the entire laying period ($p < 0.001$). Although R11 received the lowest BW among all GTs ($p < 0.001$), white GTs did not differ between each other after week 29. R11 was not affected by diet, but AA and HA fed R11 achieved higher BW compared to WLA from week 34 to 41. In general, the deficit Arg group had the lowest BW ($p < 0.001$), but HPGTs showed no difference between each other until week 29. In contrast to AA and HA, LA fed WLA reached higher BW from week 30 to 41, whereas AA and HA induced higher BW in L68 until week 33 and until week 41 in BLA ($p < 0.001$).

Furthermore, DFI was influenced by GT, diet and age as well as the interactions of GT x diet, GT x age and GT x diet x age ($p < 0.001$). In general, brown hens consumed more feed than white ones ($p < 0.001$), and BLA and R11 showed highest DFI within their phylogenetic groups. All GTs dropped their DFI continuously after maximum DFI in week 29 (HPGTs) or in week 33 (LPGTs; $p < 0.001$). Until week 25 WLA consumed more feed than R11, but this condition became reversed until the end of trial. DFI of all GTs except R11 was affected by the diets ($p < 0.001$), because increasing amounts of dietary Arg induced DFI decrease in BLA and DFI increase in L68. Consequently, LA caused a higher DFI in BLA than in L68, and AA and HA were more fed by L68 than by BLA ($p < 0.001$). Additionally, WLA consumed more AA than HA ($p < 0.01$).

Moreover, high performing WLA (week 21) and BLA (week 22) reached the onset of laying, defined by the first egg laid, at first. L68 averagely started to lay in the 23rd week of age and R11 two weeks later. Due to the different age at onset of laying, laying intensity (LI), daily egg mass (DEM) and feed to egg mass ratio (FEM) showed large differences between and within GTs in the first four weeks of trial.

LI was significantly affected by GT, diet and age as well as the interaction of GT x age. During the entire trial, the LI did not differ between HPGTs, whereas LPGTs differed
significantly between each other and from HP**GT**s until week 25 ($p < 0.001$). From week 26 to 29 **GT**s except R11 achieved their maximum LI and decreased strongly afterwards ($p < 0.001$). However, R11 reached its maximum LI four weeks later and their LI decreased strongly in the following, too. After week 30 HP**GT**s decreased so strong in LI that they even undercut the LI of L68 ($p < 0.01$). In the following, **GT**s except L68 did not differ between each other anymore. In general, the deficit groups showed lower LI than AA and HA ($p < 0.05$), occasionally.

Total EW was influenced by **GT**, diet and age as well as their interactions ($p < 0.001$). Brown eggs were heavier than white ones ($p < 0.001$), and HP**GT**s laid heavier eggs than LP**GT**s until week 33 ($p < 0.001$). From week 29 onwards L68 and R11 differed from each other ($p < 0.001$). All **GT**s except BLA showed an EW increase until week 41 ($p < 0.001$), whereas EW of BLA increased until week 33 and decreased afterwards. Until week 29 AA fed WLA and L68 differed from each other ($p < 0.001$), but they received same weights onwards. L68’s EW was statistically not affected by diet. In contrast to LA and HA, AA caused higher EW in WLA in week 29, in BLA in the weeks 25 and 37, and in R11 in week 25 ($p < 0.001$).

The calculated DEM was affected by **GT**, diet and age as well as the interaction of **GT** x age ($p < 0.01$). BLA achieved the highest DEM and differed from LP**GT**s ($p < 0.001$). WLA and L68 had higher DEM than R11 ($p < 0.001$). In general, the deficit group caused the lowest DEM among all **GT**s ($p < 0.01$). In week 26 to 29 HP**GT**s achieved their maximum DEM and they decreased strongly to the end of trial ($p < 0.001$). However, LP**GT**s reached their maximum four weeks later and decreased afterwards ($p < 0.001$), too.

FEM was only affected by hens’ age ($p < 0.001$). Due to later onset of laying, LP**GT**s showed extremely high FEM and differed from that of HP**GT**s in the first four weeks of trial ($p < 0.01$). FEM decreased rapidly from week 26 to 33 and restarted to increase until the end of trial ($p < 0.001$).

During the entire trial a mortality of 4.2 % was recorded. Losses of each **GT** are listed in the following ascending order: R11 0.0 % - WLA 4.6 % - BLA 5.6 % and L68 6.5 %.
### Table 4. Effect of Arg supply and genotype on growth and laying performance from the 22nd to the 41st week of age.

<table>
<thead>
<tr>
<th></th>
<th>WLA</th>
<th>BLA</th>
<th>R11</th>
<th>L68</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, kg/hen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>1.20</td>
<td>1.40</td>
</tr>
</tbody>
</table>

| **Daily feed intake, g/hen/d** |     |     |     |     |
| week 22-25      | 83  | 81  | 80  | 105 |
| week 26-29      | 78  | 80  | 69  | 117 |
| week 30-33      | 69  | 76  | 71  | 111 |
| week 34-37      | 65  | 69  | 61  | 99  |
| week 38-41      | 60  | 71  | 58  | 97  |

| **Laying intensity, %** |     |     |     |     |
| week 22-25      | 39.0 | 60.0 | 54.4 | 32.7 |
| week 26-29      | 73.9 | 76.8 | 73.1 | 72.8 |
| week 30-33      | 58.3 | 63.2 | 58.0 | 60.4 |
| week 34-37      | 46.7 | 57.0 | 53.1 | 61.4 |
| week 38-41      | 32.8 | 46.0 | 35.7 | 46.9 |

| **Egg weight, g/egg** |     |     |     |     |
| week 22-25      | 47.3 | 47.9 | 47.4 | 48.5 |
| week 26-29      | 48.6 | 49.6 | 47.1 | 50.9 |
| week 30-33      | 47.5 | 50.3 | 48.9 | 53.5 |
| week 34-37      | 48.9 | 49.9 | 48.4 | 52.9 |
| week 38-41      | 48.7 | 48.9 | 51.5 | 52.2 |

| **Daily egg mass, g/hen/d** |     |     |     |     |
| week 22-25      | 17.4 | 28.7 | 24.9 | 15.5 |
| week 26-29      | 35.5 | 37.5 | 34.7 | 36.8 |
| week 30-33      | 28.3 | 31.5 | 28.3 | 31.6 |
| week 34-37      | 22.6 | 29.3 | 26.1 | 32.3 |
| week 38-41      | 15.8 | 23.3 | 17.4 | 24.8 |

| **Feed to egg mass ratio, kg/kg** |     |     |     |     |
| week 22-25      | 4.7  | 2.8  | 3.2  | 6.8  |
| week 26-29      | 2.2  | 2.1  | 2.0  | 3.2  |
| week 30-33      | 2.4  | 2.4  | 2.5  | 3.5  |
| week 34-37      | 2.9  | 2.4  | 2.3  | 3.0  |
| week 38-41      | 3.8  | 3.0  | 3.3  | 3.9  |

*PSEM, GT, DIET, AGE, GTx DIET, GTx AGE, DIETx AGE, n.s.*

*< 0.001, < 0.05, > 0.05*

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\( a/b \): LSMeans values with PSEM (n = 36 chicks/experimental group) in the same row with different superscripts are significantly different (\( p < 0.05 \)); n.s.: not significant
Table 5. Effect of Arg supply and genotype on egg quality of eggs\(^1\) examined at the end of each laying month.

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<th>L68</th>
<th>PSEM</th>
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<td>AA</td>
<td>HA</td>
<td>LA</td>
<td>AA</td>
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\(^1\) eggs collected on four consecutive days of n = 36 hens of each experimental group; Proportions of egg components are calculated as weight of components (yolk, shell and albumen) per weight of total egg; \(a\), \(b\) and \(c\)Means values with PSEM (n = 36 hens/experimental group) in the same row with different superscripts are significantly different (\(p < 0.05\)); n.s. = not significant
Figure 2. Graphic presentation of calculated partial utilization of dietary Arg for egg synthesis during performance trial (LSMeans±SE; n = 36).
Egg quality

Table 5 presents the results of egg quality examination. Yolk proportion of examined eggs was significantly affected by GT, diet and age as well as the interactions GT x diet and GT x age. Eggs of HPGTs contained a lower yolk proportion than eggs of LPGTs \((p < 0.001)\). The latter ones did not differ from each other, whereas WLA showed a higher yolk proportion than BLA. During the entire trial L68 eggs increased in yolk proportion \((p < 0.05)\), but those of the other GTs enlarged their yolk proportion only until week 33 and remained constant afterwards \((p < 0.001)\). In contrast to the insufficient Arg diet, HA caused higher yolk proportion in L68 in week 25 \((p < 0.05)\).

Egg shell proportion was influenced by GT and age as well as by the interaction of GT x diet, GT x age and GT x diet x age \((p < 0.001)\). In contrast to LPGTs, HPGTs showed higher shell proportion \((p < 0.001)\), and white hens laid eggs with higher shell proportion than brown hens. Until week 33 all GTs but L68 increased in shell proportion and they decreased onwards \((p < 0.001)\). However, L68 had a constant shell proportion. Egg shell proportion was not affected by diet in all GTs but WLA, whose shell proportion was elevated by AA and HA in week 33 \((p < 0.05)\).

Proportion of albumen was significantly influenced by GT, diet and age as well as GT x age, diet x age and GT x diet x age. HPGTs laid eggs with higher albumen proportion compared with LPGTs \((p < 0.001)\). BLA achieved highest albumen proportion and L68 showed the lowest proportion \((p < 0.001)\), but white GTs did not differ from each other. Until week 33 all GTs but L68 showed a decrease, and a consecutive slight re-increase up to week 41 \((p < 0.001)\). However, L68’s albumen proportion decreased continuously to the end of trial. A dietary impact on albumen proportion was only found in WLA, as AA caused a higher proportion than LA in week 33 \((p < 0.05)\).

YC was significantly influenced by GT and age as well as by two- and three-factorial interactions. LPGTs had more intense coloured yolks than HPGTs \((p < 0.001)\). YC was not solely affected by diet, but it changed age-dependently \((p < 0.001)\). The intensity of YC decreased until week 33 and slightly re-increased until week 41.

In order to estimate the partial Arg utilization for egg production, the absolute daily Arg transfer into the egg and its protein containing proportions was calculated at first. These transfer parameters (data not shown) differed significantly between GTs, diets and age in the
way DEM and the egg proportions did, because these parameters were the mathematical product of the constant Arg concentrations described by BERGQUIST (1979), the DEM and the relative weight of the corresponding egg component. The further calculated partial Arg utilization for egg synthesis (Figure 2) was affected by GT, diet and age ($p < 0.001$). The highest partial Arg utilization for the total egg and those for egg yolk and albumen were found in WLA ($p < 0.001$). BLA showed a higher partial Arg utilization for egg albumen than LPGTs ($p < 0.01$), whereas L68 achieved a higher partial Arg utilization for egg yolk compared to BLA and R11. If dietary Arg supply elevated from LA to HA, the partial Arg utilization of all egg components decreased strongly ($p < 0.001$). The partial Arg utilization for egg albumen and that for total egg reached their maximum from week 26 to 29, and that for egg yolk peaked four weeks later ($p < 0.001$).

**Discussion**

With regard to RAUW et al. (1999), VAN DER WAAIJ (2004) and MIRKENA et al. (2010), we hypothesized that selection for high production efficiency in layers caused highly adapted HPGTs, which were less capable to cope with environmental stress compared to LPGTs. Since studied GTs were considered to be well suited for the established animal model concerning the required phylogenetic (GRANEVITZE et al. 2009; LYIMO et al. 2014) and performance divergence (LIEBOLDT et al. 2015), the present study was conducted to examine interactions between these GTs and long-term graded Arg supply serving as nutritional-environmental stress on growth development and performance of diverse purebred layer lines.

During the entire trial GTs were fed with diets equivalent to 70, 100 and 200 % Arg of recommended level (NRC 1994). Long-term effects of graded Arg supply from rearing to laying were of great importance, because significant differences in BW, DWG and DFI occurred between and within GTs at the end of rearing. These Arg-induced differences might cause diverse initial conditions for hens at the beginning of laying that could serve as carry-over effect from rearing to laying.

In the last years poultry research directed its attention frequently to the strong effects of dietary Arg on chicken’s performance (KWAK et al. 1999, 2001; CHAMRUSPOLLERT et al. 2004; JAHANIAN 2009; BULBUL et al. 2013; WANG et al. 2014 a). Comparable
responses to graded dietary Arg supply were found in studied GTs, because insufficient dietary Arg induced significant growth reduction and retardation compared to an adequate or surplus Arg supply during rearing. The estimated parameters of the Gompertz equation also indicated that adequate or even surplus dietary Arg might induce higher adult BW than insufficient Arg. In addition, lowest maximum DWG and highest $t_{\text{max}}$ were determined for chicks fed with insufficient Arg diets. Such generally growth-retarding and genetically independent effects were explained by the adverse effects of dietary imbalances between Arg and Lys (D’MELLO and LEWIS 1970, 1971; JAHANIAN 2009) and Arg and Met (KESHAVARZ and FULLER 1971 a, b). Additionally, a dietary insufficient Arg supply lowered the plasma concentrations of Arg and ornithine directly and induced a lack of these metabolizable amino acids (KWAK et al. 1999, 2001). AUSTIC and CALVERT (1981), D’MELLO (1994) and KESHAVARZ and FULLER (1971 a) described the subsequently disturbed metabolic pathways of polyamine and creatine formation, which form the basis for growth retardation and reduction in poultry suffering from insufficient and imbalanced dietary Arg. As a consequence, the Arg requirement elevated and induced a secondary growth depression (D’MELLO 1994).

Furthermore, L68 showed its strongest growth with an adequate Arg supply, whereas both White Leghorn lines and BLA developed with adequate and surplus Arg equally. Possible explanations were given by the genetically determined differences in Arg utilization and metabolism. The different rapidity in feathering caused a higher Arg requirement in fast feathering GTs during early life stage (HEGSTEDT et al. 1941). For L68, a lower Arg requirement could be assumed in times of feather development, because L68 was selected for slow feathering (K 30%, k 70%). The Arg oversupply induced growth retardation in L68 might be explained by the enhanced endogenous Arg:Lys antagonism (NESHEIM 1968; KWAK et al. 2001; BALNAVE and BRAKE 2002), and revealed a higher sensitivity of L68 for high Arg:Lys ratios. On the other hand, WLA seemed to belong to those fast feathering GTs with higher Arg requirement, because they grew well and fast with adequate and oversupplied Arg, equally.

Moreover, insufficient Arg induced a strong reduction of feed intake in studied GTs during rearing. This effect was most evident in WLA and L68, whereas BLA and R11 responded only from week 5 to 8. HARPER et al. (1970) reported on anorectic effects of dietary amino
acid imbalances and D’MELLO and LEWIS (1971) described the Arg:Lys antagonism that caused avian growth depression and induced appetite reduction, secondary. However, mechanisms of appetite inhibition caused by insufficient dietary Arg were intensively examined by WANG et al. (2014 a, b) in White Pekin ducks. The authors concluded that appetite inhibition might be a consequence of hypothalamic changes in protein expression and NO involved mechanisms of appetite regulation hormones. Based on the present study we could not consider with certainty if growth reduction has been a secondary effect of reduced feed intake induced by hypothalamic biochemical changes or resulted from adverse effects of limited metabolizable Arg in growth metabolism.

In general, our study confirmed the importance of Arg for growth-promotion during the rearing period and showed comparable results to the most studies dealing with dietary Arg in poultry (CHAMRUSPOLLERT et al. 2004; JAHANIAN 2009; BULBUL et al. 2013; WANG et al. 2014 a, b). However, these studies were carried out in growing meat-type poultry such as broilers and ducks, and recent reports on dietary effects of Arg in laying hens were not existent to the best of our knowledge. In contrast to the rearing, the corresponding GTs responded very different to the fed diets during the laying period. We assumed that the carry-over effect from rearing to laying, already mentioned above, and the dietary concentration of crude protein were responsible for the varying results between the GTs and diets. Whereas chicks, growers and pullets were fed with sufficient concentrations of crude protein (NRC 1994), the calculated basal diet for hens contained a relatively low concentration of crude protein in order to generate an Arg insufficient diet for laying hens. ROLAND (1980 a, b) emphasized the negative effects of low dietary crude protein on laying performance and egg quality. On reaching final adult BW studied GTs maintained their BW and DFI constant under commercial feeding conditions (LIEBOLDT et al. 2015). In the present study the LPGTs reached this plateau phase after the 29th week of age, whereas the HPGTs showed a strong decrease in BW after the 25th week of age. That strong decrease in BW was paralleled by a similarly directed decrease in DFI and laying performance, which were adversely affected by increasing amounts of dietary Arg. The mobilisation of body mass and its associated reduction of laying performance expressed the metabolic stress HPGTs suffering from under the given plan of nutrition. In particular the carry-over effect from rearing to laying, the dietary limitation in crude protein (CHI and SPEERS 1976; ROLAND 1980 a, b)
and the early onset of laying were main factors of metabolic stress in HPGTs. However, the dietary amino acids imbalances described by D’MELLO and LEWIS (1970) and KESHAVARZ and FULLER (1971 a) seemed to play a minor role, especially in the HPGTs, because the actual effects of graded Arg supply might be masked by the factors named before. The effects of dietary Arg supply on laying hens were most obvious in LPGTs, because the BW, DFI and laying performance of R11 was not affected by diet and those of L68 even responded to insufficient dietary Arg in the same way like reared L68. That led to the assumption that differences found in LPGT laying hens were stronger influenced by the different grades of dietary Arg as by the relatively low dietary crude protein. Because present LPGT hens showed a comparable performance to those under commercial feeding conditions (LIEBOLDT et al. 2015), the required dietary crude protein was nearly met by the formulated hen diets in order to achieve their genetically determined performance potential.

Although egg yolk contained physiologically higher amounts of Arg than egg albumen (BERGQUIST 1979), our examination of egg quality did not indicate that insufficient Arg or crude protein supply caused lower proportions of yolk and higher ones of albumen. ROLAND (1980 a, b), LEESON (1993), and HUSSEIN and HARMS (1994) reported on the virtual insensitivity of protein and amino acid deposition to dietary manipulation in eggs and meat and emphasized the genetic determination of these egg nutrients. The present study confirmed that the yolk protein synthesis was not selectively restricted by an insufficient Arg or crude protein supply. Studied laying hens responded to the adverse dietary effects by a general reduction of total egg weight and occasional shifts in egg proportions in order to reduce the loss of required amino acids into the egg. However, the amount of calculated daily transferred Arg into egg showed no differences between offered diets. The calculated partial Arg utilization for egg production reflected the intended graduation of dietary Arg, because the highest Arg utilization occurred in the insufficiently supplied hens. On reaching maximum Arg transfer into egg, a further Arg supplementation was not benefit for the synthesis of egg proteins. Consequently, the Arg not used for the synthesis of egg proteins in adequate and oversupplied diets became available for other metabolic pathways in hen’s body. Perhaps those large amounts of metabolizable Arg might also exacerbate the endogenous Arg:Lys antagonism and enhanced the adverse effects on BW and performance of laying hens as described above.
Finally, MIRKENA et al. (2010) classified the ability of reducing metabolism and performance in order to allocate not used nutrients to fitness associated traits like a stable BW as an advantage in times of poor feed quality. If GTs were unable to respond in that way, BEILHARZ et al. (1993) and RAUW et al. (1998) rated that inability as undesirable side-effect of selection on high production efficiency. If more nutrients were required for production related traits, they were taken away from fitness and allocated to production traits. Consequently, metabolic stress accompanied with decreased health, fertility and energy available for maintenance would occur with negative effects on reproduction and probability of survival (VAN DER WAAJI 2004). The established animal model of the present study seemed to be well suited for approaching that theory from an experimental point of view. The current results suggested that selection on high production efficiency caused HPGTs that were less able to cope with insufficient nutritional conditions in contrast to LPGTs.

In conclusion, growth and feed intake depressive properties of insufficient dietary Arg have been clearly shown in growing birds. The nutritional-environmental stress induced by strong limitations of Arg and crude protein in laying hens revealed HPGTs inability of simultaneous performance reduction and BW maintenance. Therefore, further and more detailed studies should be carried out in order to examine the metabolic and even immunological response of GTs to dietary limitations.

Acknowledgements
The authors gratefully acknowledge Karsten Knop and his staff for the care of the experimental animals, the sample and data collection and practical realization of experiments as well as Annerose Junghans for her participation at sample preparation.

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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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Journal of Poultry Science (JPS)

In press

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Abstract
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, L-arginine, chicken, genotype, organ growth, rearing

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. 2014 a; LIEBOLDT et al. 2015 b).

In contrast to mammals, chickens are unable to synthesise Arg de novo due to a lack of urea cycle key enzymes (TAMIR and RATNER 1963). Therefore, chickens are highly dependent 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. 2015 b) 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. 2014 b) 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 a) 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 a) 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 a, b). 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 a), 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. (2015 b). 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] x 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 concentration 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).

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

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Chicks and growers (g/kg diet)</th>
<th>Growers and pullets (g/kg diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LA</td>
<td>AA</td>
</tr>
<tr>
<td>Barley</td>
<td>200.0</td>
<td>200.0</td>
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<tr>
<td>Wheat</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Triticale</td>
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<td>-</td>
</tr>
<tr>
<td>Corn</td>
<td>399.0</td>
<td>396.0</td>
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<tr>
<td>Corn gluten meal</td>
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<td>150.0</td>
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<tr>
<td>Lucerne pellets</td>
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<td>50.0</td>
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<tr>
<td>Wheat bran</td>
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<td>39.8</td>
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<td>Soybean oil</td>
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<td>10.0</td>
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<tr>
<td>Calcium carbonate</td>
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<td>-</td>
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<td>Calcium phosphate</td>
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<tr>
<td>Premix 1</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Premix 2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-lysine HCl</td>
<td>4.9</td>
<td>4.9</td>
</tr>
<tr>
<td>L-arginine</td>
<td>-</td>
<td>3.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.0</td>
<td>3.0</td>
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</table>

<table>
<thead>
<tr>
<th>Chemical composition, g/kg diet</th>
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<tbody>
<tr>
<td>Dry matter 3</td>
</tr>
<tr>
<td>Crude ash 3</td>
</tr>
<tr>
<td>Crude protein 4</td>
</tr>
<tr>
<td>Kjeldahl Nitrogen 3</td>
</tr>
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<td>Crude fat 4</td>
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<tr>
<td>Crude fibre 3</td>
</tr>
<tr>
<td>Starch 3</td>
</tr>
<tr>
<td>Sucrose 3</td>
</tr>
<tr>
<td>Phosphorous 5</td>
</tr>
<tr>
<td>Calcium 5</td>
</tr>
<tr>
<td>AME (MJ/kg) 3</td>
</tr>
<tr>
<td>Methionine 4</td>
</tr>
<tr>
<td>Lysine 6</td>
</tr>
<tr>
<td>Arginine 4</td>
</tr>
</tbody>
</table>

1 Premix – chicks: feed additives (per kg premix): Vitamin A, 1,200,000 IU; Vitamin D₃, 350,000 IU; Vitamin E, 4,000 mg; Vitamin B₁₂, 250 mg; Vitamin B₆, 800 mg; Vitamin B₉, 600 mg; Vitamin B₁₃, 3,200 µg; Vitamin K₁, 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 D₃, 200,000 IU; Vitamin E, 2,500 mg; Vitamin B₁₂, 250 mg; Vitamin B₆, 500 mg; Vitamin B₉, 400 mg; Vitamin B₁₃, 1,850 µg; Vitamin K₁, 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 (AMEₐ), 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
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). 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 ($p_{\text{age}} < 0.001$) and L68 achieved higher BW and DWG than the other genotypes from week 6 onwards (BW: $p_{\text{genotype}} < 0.001$; DWG: $p_{\text{genotype}} < 0.05$). Because DWG of BLA did not change during the entire trial, white genotypes gained higher BW than BLA from week 6 to 12 ($p_{\text{genotype*age}} < 0.001$). 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 ($p_{\text{diet}} = 0.625$), LA fed chicks reached lower absolute BW than those fed with AA and HA ($p_{\text{diet}} < 0.05$). 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 ($p_{\text{genotype}} < 0.001$). Although DFI increased age-dependently ($p_{\text{age}} < 0.001$; $p_{\text{genotype*age}} < 0.001$), 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 ($p_{\text{diet}} < 0.01$), whereas LA even tended to induce lower DFI than AA from week 6 onwards ($p_{\text{diet*age}} = 0.077$).

Besides, the FCR was only affected by “age” ($p_{\text{age}} < 0.01$). While the first and second six-week-interval did not differ between each other, the FCR increased significantly from week 12 to 18 ($p < 0.01$).
Table 2. Effects of genotype and L-arginine supply on growth parameters from hatch to week 18.

<table>
<thead>
<tr>
<th></th>
<th>WLA</th>
<th>BLA</th>
<th>R11</th>
<th>L68</th>
<th>ANOVA (p values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LA</td>
<td>AA</td>
<td>HA</td>
<td>LA</td>
<td>AA</td>
</tr>
<tr>
<td>Body weight, g/chick</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>hatch</td>
<td>33&lt;sup&gt;D&lt;/sup&gt;</td>
<td>35&lt;sup&gt;D&lt;/sup&gt;</td>
<td>38&lt;sup&gt;D&lt;/sup&gt;</td>
<td>37&lt;sup&gt;D&lt;/sup&gt;</td>
<td>37&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td>week 6</td>
<td>293&lt;sup&gt;C,b&lt;/sup&gt;</td>
<td>368&lt;sup&gt;C,ab&lt;/sup&gt;</td>
<td>344&lt;sup&gt;C,b&lt;/sup&gt;</td>
<td>301&lt;sup&gt;C,b&lt;/sup&gt;</td>
<td>338&lt;sup&gt;C,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>week 12</td>
<td>762&lt;sup&gt;B,d&lt;/sup&gt;</td>
<td>855&lt;sup&gt;B,c&lt;/sup&gt;</td>
<td>930&lt;sup&gt;B,b&lt;/sup&gt;</td>
<td>645&lt;sup&gt;B,e&lt;/sup&gt;</td>
<td>691&lt;sup&gt;B,e&lt;/sup&gt;</td>
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<tr>
<td>week 18</td>
<td>957&lt;sup&gt;A,ef&lt;/sup&gt;</td>
<td>1034&lt;sup&gt;A,d&lt;/sup&gt;</td>
<td>1104&lt;sup&gt;A,c&lt;/sup&gt;</td>
<td>895&lt;sup&gt;A,f&lt;/sup&gt;</td>
<td>941&lt;sup&gt;A,ef&lt;/sup&gt;</td>
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<tr>
<td>Daily weight gain, g/chick/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hatch to week 6</td>
<td>6.2&lt;sup&gt;B,ab&lt;/sup&gt;</td>
<td>7.9&lt;sup&gt;B,ab&lt;/sup&gt;</td>
<td>7.3&lt;sup&gt;B,ab&lt;/sup&gt;</td>
<td>6.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>week 6 to 12</td>
<td>11.2&lt;sup&gt;A,bc&lt;/sup&gt;</td>
<td>11.6&lt;sup&gt;A,bc&lt;/sup&gt;</td>
<td>13.9&lt;sup&gt;A,ab&lt;/sup&gt;</td>
<td>8.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>week 12 to 18</td>
<td>4.6&lt;sup&gt;B,b&lt;/sup&gt;</td>
<td>4.3&lt;sup&gt;B,bc&lt;/sup&gt;</td>
<td>4.1&lt;sup&gt;B,bc&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Daily feed intake, g/chick/d</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hatch to week 6</td>
<td>19.6&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>21.6&lt;sup&gt;C,b&lt;/sup&gt;</td>
<td>22.5&lt;sup&gt;C,ab&lt;/sup&gt;</td>
<td>22.1&lt;sup&gt;C,ab&lt;/sup&gt;</td>
<td>20.8&lt;sup&gt;C,b&lt;/sup&gt;</td>
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<tr>
<td>week 6 to 12</td>
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<td>53.8&lt;sup&gt;B,b&lt;/sup&gt;</td>
<td>52.1&lt;sup&gt;B,bc&lt;/sup&gt;</td>
<td>49.6&lt;sup&gt;B,c&lt;/sup&gt;</td>
<td>52.0&lt;sup&gt;B,bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>week 12 to 18</td>
<td>68.5&lt;sup&gt;A,cd&lt;/sup&gt;</td>
<td>70.0&lt;sup&gt;A,cd&lt;/sup&gt;</td>
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<td>65.4&lt;sup&gt;A,cd&lt;/sup&gt;</td>
<td>65.4&lt;sup&gt;A,cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feed conversion ratio, g/g</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>hatch to week 6</td>
<td>3.16&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.73&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.08&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.51&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.89&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>week 6 to 12</td>
<td>4.44&lt;sup&gt;B&lt;/sup&gt;</td>
<td>4.64&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.73&lt;sup&gt;B&lt;/sup&gt;</td>
<td>6.05&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>6.19&lt;sup&gt;AB&lt;/sup&gt;</td>
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<tr>
<td>week 12 to 18</td>
<td>14.89&lt;sup&gt;A&lt;/sup&gt;</td>
<td>16.28&lt;sup&gt;A&lt;/sup&gt;</td>
<td>16.98&lt;sup&gt;A&lt;/sup&gt;</td>
<td>10.90&lt;sup&gt;A&lt;/sup&gt;</td>
<td>10.90&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

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

<sup>a-f</sup>: LSMeans values with PSEM (n = 3 chicks/group) in the same row without common superscripts differ significantly (p < 0.05)
**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.

![Figure 1](image.png)

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

<table>
<thead>
<tr>
<th>Organ</th>
<th>Heart</th>
<th>Liver</th>
<th>Pancreas</th>
<th>Gizzard</th>
<th>Spleen</th>
<th>Thymus</th>
<th>Bursa</th>
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<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>R(^2)</td>
<td>RSD</td>
<td>a (x, 10^4)</td>
<td>b</td>
<td>R(^2)</td>
</tr>
<tr>
<td>LA</td>
<td>0.015</td>
<td>0.844</td>
<td>0.900</td>
<td>0.45</td>
<td>0.193</td>
<td>0.717</td>
<td>0.966</td>
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<td>WLA</td>
<td>0.021</td>
<td>0.809</td>
<td>0.920</td>
<td>1.57</td>
<td>0.112</td>
<td>0.796</td>
<td>0.976</td>
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<tr>
<td>HA</td>
<td>0.019</td>
<td>0.822</td>
<td>0.960</td>
<td>1.13</td>
<td>0.188</td>
<td>0.721</td>
<td>0.965</td>
</tr>
<tr>
<td>LA</td>
<td>0.019</td>
<td>0.828</td>
<td>0.987</td>
<td>0.52</td>
<td>0.201</td>
<td>0.692</td>
<td>0.973</td>
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<tr>
<td>BLA</td>
<td>0.033</td>
<td>0.722</td>
<td>0.952</td>
<td>0.70</td>
<td>0.079</td>
<td>0.833</td>
<td>0.973</td>
</tr>
<tr>
<td>HA</td>
<td>0.013</td>
<td>0.877</td>
<td>0.985</td>
<td>0.60</td>
<td>0.115</td>
<td>0.776</td>
<td>0.986</td>
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<td>LA</td>
<td>0.034</td>
<td>0.728</td>
<td>0.964</td>
<td>0.89</td>
<td>0.174</td>
<td>0.719</td>
<td>0.956</td>
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<tr>
<td>R11</td>
<td>0.041</td>
<td>0.685</td>
<td>0.965</td>
<td>0.81</td>
<td>0.149</td>
<td>0.735</td>
<td>0.956</td>
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<td>HA</td>
<td>0.039</td>
<td>0.702</td>
<td>0.918</td>
<td>1.36</td>
<td>0.133</td>
<td>0.754</td>
<td>0.960</td>
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<td>LA</td>
<td>0.063</td>
<td>0.638</td>
<td>0.905</td>
<td>1.72</td>
<td>0.248</td>
<td>0.667</td>
<td>0.962</td>
</tr>
<tr>
<td>L68</td>
<td>0.013</td>
<td>0.877</td>
<td>0.964</td>
<td>1.24</td>
<td>0.051</td>
<td>0.891</td>
<td>0.966</td>
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</tbody>
</table>
| 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    | 0.038 | 0.933 | 0.943  | 0.63    | 0.834 | 0.888 | 0.971  | 0.70    | 0.011 | 0.816 | 0.885  | 1.37    

\(^1\) \(y(BW) = a \times BW^b\) 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; R2 = coefficient of determination; RSD = residual standard deviation.
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 ($p_{\text{genotype}} < 0.001; p_{\text{genotype} \times \text{age}} < 0.001$). After hatch both proportions decreased continuously in all genotypes ($p_{\text{age}} < 0.001; p_{\text{genotype} \times \text{age}} < 0.001$). From week 6 to 18 WLA had the highest liver proportion among genotypes ($p_{\text{genotype}} < 0.001; p_{\text{genotype} \times \text{age}} < 0.001$), 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 ($p_{\text{diet}} = 0.704$), LA caused higher liver proportions than AA and HA ($p_{\text{diet}} < 0.01$).

Furthermore, high performing genotypes showed higher relative gizzard weights than low performing ones at hatch ($p_{\text{genotype} \times \text{age}} < 0.001$). Afterwards white genotypes exhibited larger gizzard proportions than BLA ($p < 0.001$), which decreased continuously until the end of trial ($p_{\text{age}} < 0.001; p_{\text{genotype} \times \text{age}} < 0.001; p_{\text{diet} \times \text{age}} < 0.05$). 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 ($p_{\text{genotype}} < 0.05$). After hatch relative pancreas weight increased in genotypes except for L68, peaked at week 6 and decreased slightly until the end of trial ($p_{\text{age}} < 0.001; p_{\text{genotype} \times \text{age}} < 0.001$). 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 ($p_{\text{diet}} = 0.076$).

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: $-0.422, p < 0.001$).
<table>
<thead>
<tr>
<th>Heart, %</th>
<th>WLA</th>
<th>BLA</th>
<th>R11</th>
<th>L68</th>
<th>ANOVA (p values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatch</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LA</td>
<td>0.84, B,b</td>
<td>0.85, A,b</td>
<td>0.75, A,b</td>
<td>0.78, A,b</td>
<td>0.97, A,a</td>
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<tr>
<td>AA</td>
<td>0.44, B,ab</td>
<td>0.46, C,ab</td>
<td>0.52, B,ab</td>
<td>0.59, B,ab</td>
<td>0.51, C,ab</td>
</tr>
<tr>
<td>HA</td>
<td>0.55, B,ab</td>
<td>0.64, B,C,a</td>
<td>0.60, B,ab</td>
<td>0.64, B,ab</td>
<td>0.56, B,a</td>
</tr>
<tr>
<td>Week 6</td>
<td>0.60, B,ab</td>
<td>0.65, B,ab</td>
<td>0.65, AB,b</td>
<td>0.62, B,ab</td>
<td>0.69, B,ab</td>
</tr>
<tr>
<td>Week 12</td>
<td>0.55, B,ab</td>
<td>0.64, B,C,a</td>
<td>0.60, B,ab</td>
<td>0.64, B,ab</td>
<td>0.59, B,ab</td>
</tr>
<tr>
<td>Week 18</td>
<td>0.51, B,ab</td>
<td>0.53, C,ab</td>
<td>0.48, C,ab</td>
<td>0.56, B,a</td>
<td>0.51, C,ab</td>
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<tr>
<td>Liver, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 6</td>
<td>3.74, B,a</td>
<td>3.42, B,ab</td>
<td>3.56, A,ab</td>
<td>3.46, A,b</td>
<td>3.45, B,ab</td>
</tr>
<tr>
<td>Week 12</td>
<td>3.26, C,a</td>
<td>2.90, C,ab</td>
<td>3.08, B,ab</td>
<td>2.99, B,ab</td>
<td>3.03, C,b</td>
</tr>
<tr>
<td>Week 18</td>
<td>2.62, D,a</td>
<td>2.65, C,a</td>
<td>2.50, C,ab</td>
<td>2.34, C,b</td>
<td>2.30, D,b</td>
</tr>
<tr>
<td>Pancreas, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hatch</td>
<td>0.16, C,ab</td>
<td>0.21, C,a</td>
<td>0.16, B,ab</td>
<td>0.17, B,ab</td>
<td>0.18, C,ab</td>
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<tr>
<td>Week 6</td>
<td>0.42, A,a</td>
<td>0.40, A,b</td>
<td>0.38, A,b</td>
<td>0.34, A,b</td>
<td>0.35, A,b</td>
</tr>
<tr>
<td>Week 12</td>
<td>0.29, B,ab</td>
<td>0.30, A,b</td>
<td>0.23, B,b</td>
<td>0.32, A,b</td>
<td>0.30, A,b</td>
</tr>
<tr>
<td>Week 18</td>
<td>0.21, B,C</td>
<td>0.21, C</td>
<td>0.23, B</td>
<td>0.23, B</td>
<td>0.23, B</td>
</tr>
</tbody>
</table>

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

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-B: 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 genotypes and L-arginine supply on lymphoid organ growth from hatch to week 18.

<table>
<thead>
<tr>
<th></th>
<th>WLA</th>
<th>BLA</th>
<th>R11</th>
<th>L68</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LA</td>
<td>AA</td>
<td>HA</td>
<td>LA</td>
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<td></td>
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<tr>
<td>Bursa, %</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>hatch</td>
<td>0.19, a</td>
<td>0.15, ab</td>
<td>0.12, ab</td>
<td>0.00, b</td>
</tr>
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<td>week 6</td>
<td>0.48, A, a</td>
<td>0.51, A, a</td>
<td>0.50, A, a</td>
<td>0.28, A, c</td>
</tr>
<tr>
<td>week 12</td>
<td>0.51, A, a</td>
<td>0.45, AB, a</td>
<td>0.45, A, a</td>
<td>0.31, AB, bc</td>
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<tr>
<td>week 18</td>
<td>0.38, B, a</td>
<td>0.37, B, a</td>
<td>0.32, B, ab</td>
<td>0.26, A, bc</td>
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<tr>
<td>Thymus, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hatch</td>
<td>0.40, C, ab</td>
<td>0.45, B, a</td>
<td>0.40, C, ab</td>
<td>0.29, B, bc</td>
</tr>
<tr>
<td>week 6</td>
<td>0.53, B, b</td>
<td>0.66, A, a</td>
<td>0.63, A, a</td>
<td>0.34, AB, d</td>
</tr>
<tr>
<td>week 12</td>
<td>0.63, A, a</td>
<td>0.65, A, a</td>
<td>0.67, A, a</td>
<td>0.25, B, c</td>
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<tr>
<td>week 18</td>
<td>0.47, BC, a</td>
<td>0.53, B, a</td>
<td>0.51, B, a</td>
<td>0.39, A, b</td>
</tr>
<tr>
<td>Spleen, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hatch</td>
<td>0.10, B, a</td>
<td>0.06, C, b</td>
<td>0.04, C, b</td>
<td>0.05, B, b</td>
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<tr>
<td>week 6</td>
<td>0.22, A, bc</td>
<td>0.23, A, b</td>
<td>0.23, A, b</td>
<td>0.18, A, c</td>
</tr>
<tr>
<td>week 12</td>
<td>0.19, A, c</td>
<td>0.18, B, c</td>
<td>0.18, B, c</td>
<td>0.20, A, bc</td>
</tr>
<tr>
<td>week 18</td>
<td>0.20, A, bc</td>
<td>0.17, B, c</td>
<td>0.19, AB, bc</td>
<td>0.22, A, b</td>
</tr>
</tbody>
</table>

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)
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 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. 2014 a, b; LIEBOLDT et al. 2015 b).

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-dependent 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. 2014 b). 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. 2014 a, b). 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. 2015 b). 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 a, b) 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 a, b), 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 (CIRIAOC 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. (1981 a, b) and DALY et al. (1990) have described beneficial effects of dietary Arg supplementation in mammals with increasing thymus weight and cellularity. DORSHKIND and HORSEMAN (2000) and CALDER and YAQQOOb (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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Haematological and Febrile Response to *Escherichia coli* Endotoxin in 12-week-old Cockerels of Genetically Diverse Layer Lines Supplied with Graded Dietary L-arginine

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*Journal of Animal Physiology and Animal Nutrition*

Submitted
Summary

Due to its decisive functions in metabolic, endocrine and immune system L-arginine (Arg) is dietary indispensable for chickens. In 12-week-old genetically diverse cockerels effects of \textit{ad libitum} graded Arg supply on haematological and febrile response were studied over 48 hours after single lipopolysaccharide (LPS) injection. Offered diets contained Arg equivalent to 70, 100 and 200 \% of recommended Arg supply. Pathophysiological alterations in weight gain, feed intake, body temperature and differential blood count were examined in comparison with physiological initial values.

Within the first 24 hours after LPS injection cockerels reduced feed intake and lost body weight subsequently. Thereby, low performing genotypes lost body weight to a lesser extent than high performing ones. The loss of body weight was further intensified by deficient dietary Arg. Within the following 24 hours cockerels recovered from LPS by improving feed intake and weight gain. Furthermore, LPS induced genotype-specific fever response: Brown genotypes showed initial hypothermia followed by longer-lasting moderate hyperthermia on the one hand and white genotypes exhibited biphasic hyperthermia on the other hand. Fever response was accompanied by significant changes in differential blood counts. Severe leukopenia, characterised by lymphopenia and heterophilia, was observed from 4 to 8 hours after LPS injection, and replaced by a marked leukocytosis with longer-lasting monocytosis up to 48 hours after LPS injection. Under given pathophysiological conditions deficiently Arg supplied cockerels had higher total leukocyte counts than cockerels supplied with adequate and surplus Arg. Deficient and surplus dietary Arg even tended to cause higher ratios between heterophils and lymphocytes.

To conclude, present results confirmed that LPS induced numerous immunological changes in 12-week-old cockerels and emphasized that chicken genotype is a source of variation to be considered for immunological studies. A lack of essential dietary Arg intensified acute changes in differential blood counts and weight gain during LPS-induced inflammation.

\textbf{Keywords:} anorexia, differential blood count, genotype, heterophils to lymphocytes ratio, hyperthermia, hypothermia
**Introduction**

As a result of increasing pressure from consumers and legislative the general prophylactic and therapeutic usage of dietary antibiotics are widely excluded from poultry diets in many parts of the world (BEDFORD 2000; GRAHAM et al. 2007). Alternatively the concept of nutritional immunomodulation is more in the focus during the past years. KORVER (2012) defined this concept as the targeted supplementation of specific dietary nutrients to alter certain aspects of immune function. The basic amino acid L-arginine (Arg) belongs to these specific dietary nutrients (KWAK et al. 1999; LEE et al. 2002; TAN et al. 2014). Due to birds’ lack of urea cycle key enzymes chickens are unable to synthesize Arg de novo from ornithine (TAMIR and RATNER 1963) causing a direct interrelationship between the concentrations of plasma Arg and dietary Arg (CHU and NESHEIM 1979; KWAK et al. 1999).

Arg is involved in multiple physiological processes like growth and feathering, and plays a decisive role in protein biosynthesis and serves as precursor of many metabolites (reviewed in: KHAJALI and WIDEMAN 2010). In the avian immune system, Arg serves as only known precursor of nitric oxides (NO), synthesized by induced nitric oxide synthase (iNOS). This free radical acts as paracrine immune mediator and cytotoxic product of activated avian thrombocytes (ST. PAUL et al. 2012) and macrophages (QURESII 2003; BOWEN et al. 2007) whose NO production is substrate-limited by Arg (SUNG et al. 1991; KIDD et al. 2001). Depending on its secretagogue activities and its role as precursor of polyamines, Arg modulates lymphoid organ development, proportions of peripheral blood leukocytes as well as proportions of T cell subpopulations after immune challenge further (KWAK et al. 1999; LEE et al. 2002; TAYADE et al. 2006; MUNIR et al. 2009; TAN et al. 2014).

In order to induce an acute phase response in chicken, lipopolysaccharide (LPS) from cell wall of Gram-negative bacteria is frequently used as immune stimulator (XIE et al. 2000; LESHCHINSKY and KLASING 2001; CHENG et al. 2004; TAN et al. 2014). The following systemic inflammation is coordinated by the release of NO and avian equivalents of pro-inflammatory cytokines IL-1β, IL-6 and tumour-necrosis-factor (TNF)-α from activated avian leukocytes (KLASING and PENG 1990; DIL and QURESII 2002 a; FARNELL et al. 2003). As a result, unspecific sickness behaviour characterised by anorexia and lethargy as well as increased hepatic secretion of acute phase proteins, alterations of body temperature and
Peripheral blood leukocyte proportions occur in challenged chickens (Xie et al. 2000; Leshchinsky and Klasing 2001; Cheng et al. 2004). During acute phase response in porcine (Bruins et al. 2002, Luijking et al. 2005) and rodent sepsis (Nirgiotis et al. 1991; Milakofsky et al. 1993) the plasma availability of Arg decreases, while enteral Arg supply can be particularly advisable for the outcome of this process (Suchner et al. 2002; Li et al. 2007).

With reference to Kwak et al. (2001) and Van Eerden et al. (2004) showing genetically dependent differences in Arg requirement and immune response of different Cornell K strains as well as differences in antibody response between low and highly efficient pullets, we hypothesized that layer strains with different production efficiency and supplied with graded dietary Arg may respond immunologically different to LPS-induced systemic inflammation. In order to get a deeper insight in the interaction of genetic background and dietary environment on chicken’s metabolic and immunological response, we have further developed the animal model described by Lieboldt et al. (2015 a). Because rearing period exposes layer-type birds to various stressors influencing growth, metabolism and immunity as well as later production efficiency, our objective was to examine the haematological and febrile response in 12-week-old cockerels of four purebred layer lines adapted to three levels of dietary Arg suffering from experimentally induced acute phase response.

Materials and Methods

**Birds, husbandry and experimental diets**

The present study was carried out with twelve 12-week-old layer-type cockerels (Gallus gallus domesticus) of four purebred layer lines each. These lines comprised two commercial high performing genotypes (WLA and BLA) from Lohmann Tierzucht GmbH, Cuxhaven, Germany and two low performing ones (R11 and L68) from non-selected resource populations at the Institute of Farm Animal Genetics, Neustadt-Mariensee, Germany (Lieboldt et al. 2015 a). Both white layer lines (WLA and R11) were of White Leghorn origin and phylogenetically closely related, but distant from the Rhode Island Red line BLA and its counterpart L68 (New Hampshire). Cockerels of the present study were reared under the same conditions described in Lieboldt et al. (2015 b) for layer pullets. After hatch birds 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 (NATIONAL RESEARCH COUNCIL, NRC 1994) from hatch to week 7 and from week 8 to 16, respectively.

In the present study birds were housed in metabolic single-cages (42 cm x 35 cm x 42 cm) of a three-floor battery equipped with outside feed trough and water bowl in an environmentally controlled room for 10 days. During the experiment temperature was between 18 to 19 °C and light period lasted from 0600 to 1600 h (10L:14D). Feed and water were provided *ad libitum*. The 12 cockerels of each genotype were fed further with the three diets equivalent to 70, 100 and 200 % of recommended age-specific Arg level (Table 1) with 4 replicate cages. Cockerels were fed with a low Arg containing basal diet (LA; 4.74 g Arg/kg diet) that was further supplemented to adequate (AA; 6.46 g Arg/kg diet) and high Arg (HA; 13.44 g Arg/kg diet) by adding free Arg base (99 %, Europepta, Hannover, Germany) at the expense of corn. The basal diet was also supplemented with any deficient essential amino acid other than Arg.

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).

*Experimental procedure and sample collection*

After 7 days of adaptation to environmental conditions the actual experiment was carried out in cockerels’ 12th week of age and lasted 3 days. Depending on their genetic background and long-term graded Arg supply during the preceding period, initial body weight varied between genotypes. R11 (755 ± 30 g/chick) and L68 (1040 ± 30 g/chick) differed significantly from WLA (910 ± 30 g/chick), BLA (920 ± 30 g/chick) and between each other. Thereby, cockerels reared with LA weighed averagely 80 g/chick less than AA and HA fed birds.

The actual experiment was subdivided into two parts: examination of physiological conditions at day 1 and LPS-induced pathophysiological conditions over 48 hours at days 2 and 3. On day 1 the trial began at 0730 h after recording body weight. At 0730 h on day 2 cockerels’ body weight was recorded again and all birds were challenged with 2 mg *Escherichia coli* (E.coli) LPS per kg body weight (serotype O111:B4, Sigma Aldrich Chemie GmbH, Munich, Germany), dissolved at 2 mg LPS in 1 mL sterile saline solution (0.9 % NaCl, B. Braun
Melsungen AG, Melsungen, Germany). LPS was injected into the left pectoral muscle. In this study separate saline control groups were omitted because LPS-induced alterations were compared with their physiological baselines in cockerels to account for bird’s individual variability in examined clinical traits. Body weight of cockerels was recorded at 24 and 48 hours post injectionem (p. inj.) further and residual feed was recorded daily. To consider genotype-dependent differences in growth associated parameters the calculated daily weight gain (DWG) and daily feed intake (DFI) were raised to the power of 0.75, i.e. the metabolic body weight (kg BW^{0.75}).

From one hour before treatment to 28 hours p. inj. rectal body temperature (RBT) was manually recorded with a digital thermometer (Veterinär-Thermometer SC 12, Scala electronic GmbH, Stahnsdorf, Germany) 2 cm deep in cockerels’ rectum cranial the coprodaeum. The measuring intervals were 20 minutes (min) from one hour before injection to 7 hours p. inj., 30 min from 7 to 14 hours p. inj., 60 min from 14 to 18 hours p. inj., and 120 min from 18 to 28 hours p. inj.. Because cockerels’ RBT regained baseline values from 28 hours p. inj. onwards, further RBT examination until 48 hours p. inj. was omitted.

To prepare blood smears and determine haematocrit venous blood from the Vena basilica sinistra was collected with 0.45 x 25 mm cannula (26 gauge, Sterican®, B. Braun Melsungen AG, Melsungen, Germany) in 1 ml EDTA monovettes (Sarstedt AG & Co, Nümbrecht, Germany) before LPS injection (0 h) and at 4, 8, 24 and 48 hours p. inj..

**Preparation and analysis of blood smears**

To perform differential white blood cell (WBC) counts, blood smears were made from the EDTA blood samples and stained using Wright-Giemsa stain. Total counts of leukocytes and thrombocytes were calculated by the method of CAMPBELL and ELLIS (2007). In the following 200 leukocytes were counted using a light microscope (Zeiss, West Germany) under oil immersion at a magnification of 1000 and differentiated for lymphocytes, heterophils, eosinophils, basophils and monocytes. The ratio between heterophils and lymphocytes (H/L ratio) was calculated by division of both cell proportions further. Two smears of each cockerel and sampling time were analysed and their mean values were used for further statistical analysis. The corresponding haematocrit was determined by using heparinized capillaries after centrifugation in a micro-haematocrit centrifuge. Two capillaries
of each cockerel and sampling time were analysed. All used haematological procedures were described by PENDL (2008 a, b) in detail.

**Table 1.** Ingredient composition, analysis and calculations (g/kg diet) of low (LA), adequate (AA) and high (HA) L-arginine supplied experimental diets.

<table>
<thead>
<tr>
<th>Ingredients (g/kg diet)</th>
<th>LA</th>
<th>AA</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>300.0</td>
<td>300.0</td>
<td>300.0</td>
</tr>
<tr>
<td>Wheat</td>
<td>150.0</td>
<td>150.0</td>
<td>150.0</td>
</tr>
<tr>
<td>Triticale</td>
<td>147.5</td>
<td>147.5</td>
<td>147.5</td>
</tr>
<tr>
<td>Corn</td>
<td>209.5</td>
<td>208.0</td>
<td>201.5</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>80.0</td>
<td>80.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Lucerne pellets</td>
<td>60.0</td>
<td>60.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Vitamin-trace mineral premix*</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>L-lysine HCl</td>
<td>2.6</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>L-arginine</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
</tr>
</tbody>
</table>

**Analisled values (g/kg diet)**

<table>
<thead>
<tr>
<th></th>
<th>LA</th>
<th>AA</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>888.4</td>
<td>891.4</td>
<td>891.7</td>
</tr>
<tr>
<td>Crude ash</td>
<td>52.1</td>
<td>53.2</td>
<td>54.2</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>20.5</td>
<td>21.9</td>
<td>23.1</td>
</tr>
<tr>
<td>Crude fat</td>
<td>30.7</td>
<td>31.6</td>
<td>34.6</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>38.5</td>
<td>39.3</td>
<td>39.3</td>
</tr>
<tr>
<td>Starch</td>
<td>493.2</td>
<td>491.8</td>
<td>482.8</td>
</tr>
<tr>
<td>Sucrose</td>
<td>23.8</td>
<td>24.3</td>
<td>23.0</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>13.9</td>
<td>14.3</td>
<td>13.8</td>
</tr>
<tr>
<td>Calcium</td>
<td>11.5</td>
<td>11.8</td>
<td>11.6</td>
</tr>
</tbody>
</table>

**Calculated values (MJ/kg or g/kg diet)**

<table>
<thead>
<tr>
<th></th>
<th>LA</th>
<th>AA</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMEn†</td>
<td>11.6</td>
<td>11.7</td>
<td>11.8</td>
</tr>
<tr>
<td>Methionine‡</td>
<td>2.43</td>
<td>2.43</td>
<td>2.42</td>
</tr>
<tr>
<td>Lysine‡</td>
<td>6.26</td>
<td>6.26</td>
<td>6.24</td>
</tr>
<tr>
<td>Arginine‡</td>
<td>4.74</td>
<td>6.46</td>
<td>13.44</td>
</tr>
</tbody>
</table>

*Premix (Deutsche Vilomix Tierernährung GmbH, Neuenkirchen-Vörden, Germany) contained per kg of mixture: vitamin A, 1,000,000 IU (retinyl acetate); vitamin D₃, 200,000 IU (cholecalciferol); vitamin E, 2,500 IU (DL-α-tocopheryl-acetate); vitamin B₁, 250 mg; vitamin B₂, 500 mg; vitamin B₆, 400 mg; vitamin B₁₂, 1,850 µg; vitamin K₃, 300 mg; nicotinic acid, 3,000 mg; pantothenic acid, 900 mg; folic acid, 80 mg; biotin, 2,100 µg; choline chloride, 30,000 mg; iron from ferrous-(II)-sulphate monohydrate, 4,000 mg; copper from cupric-sulphate pentahydrate, 1,500 mg; manganese from manganese-(II)-oxide, 8,000 mg; zinc from zinc-oxide, 8,000 mg; iodine from calcium iodate, 160 mg; selenium from sodium selenite, 32 mg; cobalt from basic cobalt-(II)-carbonate monohydrate, 20 mg; Butylated hydroxy toluene, 10,000 mg.

‡Calculation according to energy estimation equation of the World’s Poultry Science Association (VOGT 1986).

§Calculation based on analysed amino acid contents of ingredients and their proportions of the diets.

**Analysis of feed**

Experimental diets (Table 1) were analysed for dry matter, Kjeldahl nitrogen, crude ash, crude fat, crude fibre, starch, sucrose, phosphorus and calcium. All analysis was in accordance to the methods of the Association of German Agricultural Analytic (VDLUFA; BASSLER...
1997). The nitrogen-corrected apparent metabolisable energy (AME\textsubscript{N}) of diets was calculated using energy estimation equation of the World’s Poultry Science Association (VOGT 1986). In order to calculate the concentrations of amino acids in the experimental diets appropriately, amino acid containing feed components others than those supplemented in their free forms were analysed for the concentrations of containing amino acids by ion exchange chromatography described in the analytical methods of AMINODat 4.0 (EVONIK INDUSTRIES 2010).

**Statistical data analysis**

Statistical evaluation was performed using the software package of SAS 9.4 (SAS INSTITUTE INC. 2012). In general, procedure MIXED was used for evaluating the data. Fixed effects were “genotype” (WLA, BLA, R11 and L68), “diet” (LA, AA and HA), and “time” (observation specific time levels) as well as their 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* time-dependent repeated structure within cockerels. As traits were measured repeatedly on the same cockerel, a “repeated” statement was considered in the statistical model to account for similarities within subjects. 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 a probability level lower or equal to 0.05. The Tukey-Kramer test was applied for a multiple comparison of means. Based on the described model the mean value differences were evaluated and presented in two different ways using the “pdiff” statement. First, for each trait the difference between the initial value before treatment and for a particular time after treatment was tested by *t*-test. Secondly, treatment differences were evaluated separately for each time. Least square means and standard errors were presented graphically along with the statistics.

**Results**

*Daily weight gain and daily feed intake*

DWG (Figure 1) and DFI (Figure 2) were affected by “genotype”, “time” and their interaction (*p* < 0.001). A single dietary effect was not found on both traits (DWG: *p*_\textsubscript{diet} = 0.667; DFI:
In general and before LPS injection brown genotypes achieved a higher DWG than white genotypes, but BLA showed lowest DFI among the four genotypes ($p_{\text{genotype}} < 0.001$; $p_{\text{genotype*time}} < 0.001$). Within the first 24 hours $p$. inj. LPS induced a strong decrease in DFI accompanied by a significant loss of body weight ($p_{\text{time}} < 0.001$; $p_{\text{genotype*time}} < 0.001$). Latter one was intensified by insufficient dietary Arg supply in contrast to both supplemented diets ($p_{\text{diet*time}} < 0.05$). Whereas DFI did not differ between cockerels in the first 24 hours $p$. inj., low performing genotypes lost body weight to a lesser extent than high performing ones ($p < 0.01$). Within the second 24 hours $p$. inj. DWG and DFI increased. While DWG exceeded its baseline value in all genotypes ($p < 0.001$), only BLA regained its baseline DFI and differed from the other cockerels ($p < 0.001$). In addition, R11 recovered in body weight to a lesser extent than the other genotypes ($p < 0.001$).

Figure 1. Time-dependent alterations in daily weight gain of 12-week-old genetically diverse purebred cockerels supplied with graded L-arginine during acute phase response (LSMeans ± SE, n = 4). Note: $a$. inj.: ante injectionem (day 1); $p$. inj.: post injectionem (days 2 and 3); 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; $^{a}$: LSMeans without a common superscript differ significantly ($p < 0.05$); ANOVA: $p_{\text{genotype}} < 0.001$; $p_{\text{diet}} = 0.667$; $p_{\text{time}} < 0.001$; $p_{\text{genotype*diet}} = 0.868$; $p_{\text{genotype*time}} < 0.001$; $p_{\text{diet*time}} < 0.01$; $p_{\text{genotype*diet*time}} = 0.201$. 
Figure 2. Time-dependent alterations in daily feed intake of 12-week-old genetically diverse purebred cockerels supplied with graded L-arginine during acute phase response (LSMeans ± SE, n = 4). Note: a. inj.: ante injectionem; p. inj.: post injectionem; 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; a-f: LSMeans without a common superscript differ significantly (p < 0.05); ANOVA: p_{genotype} < 0.001; p_{diet} = 0.660; p_{time} < 0.001; p_{genotype*diet} = 0.918; p_{genotype*time} < 0.01; p_{diet*time} = 0.597; p_{genotype*diet*time} = 0.987.

Rectal body temperature

The course of RBT during acute phase response is graphically shown in Figure 3. RBT was affected by “genotype”, “time” and their interaction (p < 0.001). A dietary effect did not occurred (p_{diet} = 0.318). Genotypes showed physiological RBT of average 41.4 ± 0.1 °C before LPS injection and did not differ from each other. However, LPS induced genotype-specific temperature profiles and amplitudes of fever (p_{genotype} < 0.001; p_{time} < 0.001; p_{genotype*time} < 0.001). RBT of brown genotypes decreased 3 hours p. inj. and led to a severe hypothermia 5 hours p. inj. (L68: 40.5 ± 0.1 °C; BLA: 40.4 ± 0.1 °C; p < 0.001). Afterwards RBT increased and remained elevated at 42.0 ± 0.1 °C (BLA) and 41.8 ± 0.1 °C (L68) from 11 to 17 hours p. inj. (p < 0.001). In the following RBT of brown genotypes slightly decreased and approached its physiological initial value of 41.4 ± 0.1 °C at 24 hours p. inj.. On the contrary, in white genotypes’ fever response missed an initial hypothermia. WLA and R11 showed a severe biphasic fever response within 2 hours p. inj. (R11: up to 43.0 °C; WLA: up to 42.8 °C) and 11 hours p. inj. (up to 42.3 °C; p < 0.001), whereby each
hyperthermic peak lasted for 2 to 4 hours. After the second peak both genotypes approached their baseline value of 41.4 ± 0.1 °C at 14 hours p. inj.

**Figure 3.** Time-dependent alterations in rectal body temperature of 12-week-old genetically diverse purebred cockerels supplied with graded L-arginine during acute phase response (LSMeans; PSEM = 0.1 °C, n = 4; a) high performing genotypes; b) low performing genotypes). **Note:** i.m.: intramuscular; LPS: lipopolysaccharide; 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; *: LSMeans of white genotypes differed significantly from their initial value (p < 0.05); **: LSMeans of brown genotypes differed significantly from their initial value (p < 0.05); ANOVA: Pgenotype < 0.001; Pdiet = 0.318; Ptime < 0.001; Pgenotype*diet = 0.213; Pgenotype*time < 0.001; Pdiet*time = 0.255; Pgenotype*diet*time = 0.657.
**Haematology**

Total leukocyte and thrombocyte counts (Figures 4 and 5) as well as H/L ratio (Figure 6) are presented over time graphically, whereas WBC proportions are summarized in Table 2. All types of WBC were influenced by “genotype”, “diet”, “time” and “genotype and time” differently.

The total leukocyte counts (Figure 4) were affected by cockerels’ genotype ($p_{\text{genotype}} < 0.001$), generally. BLA cockerels had the significantly highest total leukocyte counts ($14.9 \pm 0.5 \times 10^3$ WBC/µL), whereas R11 cockerels showed the lowest one ($9.8 \pm 0.5 \times 10^3$ WBC/µL). Low performing genotypes exhibited lower initial total leukocyte counts than their high performing counterparts ($12.3 \pm 0.9 \times 10^3$ WBC/µL vs. $9.5 \pm 0.9 \times 10^3$ WBC/µL; $p_{\text{genotype}*\text{time}} < 0.001$). Insufficient dietary Arg induced a higher total leukocyte counts than both supplemented diets ($13.4 \pm 0.4 \times 10^3$ WBC/µL vs. $12.0 \pm 0.4 \times 10^3$ WBC/µL; $p_{\text{diet}} < 0.05$). From LPS injection to 4 hours $p. \text{inj.}$ total leukocyte counts decreased strongly ($p_{\text{time}} < 0.001$), achieved a baseline plateau from 8 to 24 hours $p. \text{inj.}$ and increased further up to 48 hours $p. \text{inj.}$, where total leukocyte counts even exceeded baseline values ($p < 0.001$).

Although total thrombocyte counts (Figure 5) were not influenced nutritionally ($p_{\text{diet}} = 0.741$), “time” and “genotype and time” influenced this cell type strongly ($p < 0.001$). L68 showed lower initial total thrombocyte counts among genotypes ($25.4 \pm 1.8 \times 10^3$ thrombocytes/µL vs. $16.2 \pm 1.8 \times 10^3$ thrombocytes/µL; $p_{\text{genotype}} < 0.001$). After LPS injection total thrombocyte counts decreased and achieved a plateau from 8 to 24 hours $p. \text{inj.}$ ($p_{\text{time}} < 0.001$; $p_{\text{genotype}*\text{time}} < 0.001$). Until 48 hours $p. \text{inj.}$ thrombocytes strongly increased and exceeded their initial values in all genotypes ($p < 0.001$).

The H/L ratio (Figure 6) was also affected by “genotype”, “time” and their interaction ($p < 0.001$). BLA cockerels showed higher H/L ratios than the other genotypes, generally ($1.24 \pm 0.06$ vs. $0.77 \pm 0.06$; $p_{\text{genotype}} < 0.001$; $p_{\text{genotype}*\text{time}} < 0.001$). The insufficient and oversupplied Arg diets tended to cause higher H/L ratios than the adequate supplied Arg diet ($0.94 \pm 0.05$ vs. $0.78 \pm 0.05$; $p_{\text{diet}} = 0.068$). Before treatment genotypes did not differ from each other. However, H/L ratio increased markedly after LPS injection and peaked 4 hours $p. \text{inj.}$ in white and 8 hours $p. \text{inj.}$ in brown genotypes ($p_{\text{time}} < 0.001$; $p_{\text{genotype}*\text{time}} < 0.001$). Latter ones differed from each other and achieved higher H/L ratios than white ones at their peak ($p <$...
In the following H/L ratios decreased and regained baseline at 24 hours p. inj. in L68, R11 and WLA, and at 48 hours p. inj. in BLA. Furthermore, WBC proportions (Table 2) were significantly affected by “genotype”, “time” and their interaction, but they showed no dietary impact and the proportion of eosinophils was not affected by “genotype” additionally.

WLA and L68 cockerels had a higher proportion of lymphocytes than R11 and BLA (58.2 ± 1.3 % vs. 50.3 ± 1.3 %; \( p_{\text{genotype}} < 0.001 \)). After treatment the lymphocyte proportion strongly decreased until 4 to 8 hours p. inj. and regained baseline values at 48 hours p. inj. in all genotypes but L68 (\( p_{\text{time}} < 0.001; p_{\text{genotype}\times\text{time}} < 0.001 \)). Latter one reached its initial value at 24 hours p. inj. already and remained constant afterwards. Because heterophils’ response to LPS was exactly opposite to that of the lymphocytes and both cell types were negatively correlated (Pearson correlation coefficient: -0.983, significance: \( p < 0.001 \)) a further description of heterophils has been omitted here and details are shown in Table 2.

Moreover, the initial proportion of WLA eosinophils differed from those of the other genotypes (1.5 ± 0.1 % vs. 1.0 ± 0.1 %; \( p_{\text{genotype}} < 0.001 \)). After LPS injection eosinophils nearly disappeared from blood for 4 to 8 hours and regained initial values 48 hours p. inj. On the contrary, the proportion of basophils was lowest in BLA (\( p_{\text{genotype}} < 0.001 \)). After treatment this cell proportion decreased within 4 to 8 hours and regained its initial value except for R11 at 24 hours p. inj. and remained constant afterwards except for L68 (\( p_{\text{time}} < 0.001; p_{\text{genotype}\times\text{time}} < 0.05 \)). During the trial basophils of R11 did not regain their baseline, while those of L68 re-decreased from 24 to 48 hours p. inj. Finally, the monocyte proportion of L68 was the smallest among examined genotypes (\( p_{\text{genotype}} < 0.01 \)). In the first 4 hours p. inj. the proportion of monocytes did not respond to LPS. At 8 hours p. inj. the proportion increased and peaked at 24 hours p. inj. in white genotypes and at 48 hours p. inj. in brown genotypes (\( p_{\text{time}} < 0.001; p_{\text{genotype}\times\text{time}} < 0.001 \)). While white cockerels had a significantly higher proportion of monocytes than brown ones at 24 hours p. inj., another 24 hours later this difference disappeared.
Figure 4. Time-dependent alterations in total leukocyte count of 12-week-old genetically diverse purebred cockerels supplied with graded L-arginine during acute phase response (LSMeans ± SE, n = 4; a) high performing genotypes; b) low performing genotypes). Note: LPS: lipopolysaccharide; 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; *: LSMeans of all genotypes differed significantly from their initial value (p < 0.05); ANOVA: $p_{\text{genotype}} < 0.001; p_{\text{diet}} < 0.05; p_{\text{time}} < 0.001; p_{\text{genotype} \times \text{diet}} = 0.181; p_{\text{genotype} \times \text{time}} < 0.001; p_{\text{diet} \times \text{time}} = 0.281; p_{\text{genotype} \times \text{diet} \times \text{time}} = 0.440$. 

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Figure 5. Time-dependent alterations in total thrombocyte count of 12-week-old genetically diverse purebred cockerels supplied with graded L-arginine during acute phase response (LSMeans ± SE, n = 4; a) high performing genotypes; b) low performing genotypes). Note: LPS: lipopolysaccharide; 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; *: LSMeans of all genotypes differed significantly from their initial value ($p < 0.05$); ANOVA: $p_{\text{genotype}} = 0.623$; $p_{\text{diet}} = 0.741$; $p_{\text{time}} < 0.001$; $p_{\text{genotype*diet}} = 0.650$; $p_{\text{genotype*time}} < 0.001$; $p_{\text{diet*time}} = 0.341$; $p_{\text{genotype*diet*time}} = 0.812$. 

0 10 20 30 40 50

0 8 16 24 32 40 48

Total thrombocytes count ($\times 10^3$ cells/µL)

Hours post LPS injection

WLA LA —— WLA AA —— WLA HA

BLA LA —— BLA AA —— BLA HA

R11 LA —— R11 AA —— R11 HA

L68 LA —— L68 AA —— L68 HA

Light off Light off
Figure 6. Time-dependent alterations in heterophils to lymphocytes ratio of 12-week-old genetically diverse purebred cockerels supplied with graded L-arginine during acute phase response (LSMeans ± SE, n = 4; a) high performing genotypes; b) low performing genotypes). Note: LPS: lipopolysaccharide; 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; *: LSMeans of all genotypes differed significantly from their initial value (p < 0.05); **: only LSMeans of BLA differed significantly from their initial value (p < 0.05); ANOVA: $p_{\text{genotype}} < 0.001$; $p_{\text{diet}} = 0.068$; $p_{\text{time}} < 0.001$; $p_{\text{genotype}\times\text{diet}} = 0.211$; $p_{\text{genotype}\times\text{time}} < 0.01$; $p_{\text{diet}\times\text{time}} = 0.346$; $p_{\text{genotype}\times\text{diet}\times\text{time}} = 0.712$. 
Table 2. Time-dependent alterations in white blood cell proportions of 12-week-old genetically diverse purebred cockerels supplied with graded L-arginine during acute phase response (LSMeans, PSEM, n = 4).

<table>
<thead>
<tr>
<th>WBC</th>
<th>Hours p. LPS</th>
<th>WLA LA</th>
<th>AA</th>
<th>HA</th>
<th>BLA LA</th>
<th>AA</th>
<th>HA</th>
<th>R11 LA</th>
<th>AA</th>
<th>HA</th>
<th>L68 LA</th>
<th>AA</th>
<th>HA</th>
<th>PSEM</th>
</tr>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
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<td>78.3 A, ab</td>
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<td>58.6 A, d</td>
<td>58.8 A, d</td>
<td>69.9 A, b</td>
<td>67.8 A, bc</td>
<td>71.1 A, b</td>
<td>62.2 C, c</td>
<td>62.2 C, c</td>
<td>66.3 B, bc</td>
<td>2.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>4</td>
<td>45.3 C, ab</td>
<td>41.7 C, bc</td>
<td>34.7 C, cd</td>
<td>32.5 C, d</td>
<td>46.9 A, b</td>
<td>38.7 C, c</td>
<td>38.0 C, c</td>
<td>44.6 B, c</td>
<td>38.6 C, c</td>
<td>41.6 D, b</td>
<td>50.7 D, a</td>
<td>43.1 C, b</td>
<td>31.8 E, b</td>
<td>35.6 E, ab</td>
</tr>
<tr>
<td>8</td>
<td>37.4 D, ab</td>
<td>37.4 C, ab</td>
<td>38.4 C, a</td>
<td>21.5 D, b</td>
<td>31.1 D, b</td>
<td>26.5 D, b</td>
<td>36.6 D, b</td>
<td>38.4 D, a</td>
<td>38.7 C, a</td>
<td>31.8 E, b</td>
<td>35.6 E, ab</td>
<td>30.4 D, bc</td>
<td>29.1 C, ab</td>
<td>29.7 C, ab</td>
</tr>
<tr>
<td>24</td>
<td>59.5 B, c</td>
<td>59.1 B, c</td>
<td>60.3 B, c</td>
<td>42.4 B, c</td>
<td>53.7 R, b</td>
<td>44.7 R, b</td>
<td>50.4 B, d</td>
<td>58.3 B, ed</td>
<td>57.3 B ed</td>
<td>68.6 B, b</td>
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<td>77.6 A, ab</td>
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<td>76.4 A, ab</td>
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<td>60.9 A, d</td>
<td>57.0 A, d</td>
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<td>53.8 A, bc</td>
<td>59.0 A, bc</td>
<td>54.9 A, b</td>
<td>60.1 A, b</td>
<td>52.9 C, ab</td>
<td>48.1 C, bc</td>
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<td>54.9 A, b</td>
<td>60.1 A, b</td>
<td>21.5 D, a</td>
<td>22.3 D, d</td>
</tr>
<tr>
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<td>25.9 B, cd</td>
<td>26.2 B, cd</td>
<td>26.1 B, cd</td>
<td>48.8 B, a</td>
<td>34.3 C, bc</td>
<td>45.9 C, a</td>
<td>34.7 B, b</td>
<td>30.4 B, bc</td>
<td>28.6 B, c</td>
<td>8.9 C, de</td>
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<td>4.7 C, e</td>
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<td>0.394</td>
<td>0.101</td>
<td>0.033</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

WBC: white blood cells; LPS: E.coli endotoxin (lipopolysaccharide); WLA: high performing White Legorn; 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,b: LSMeans of one cell type within the same column without a common superscript differ significantly (p < 0.05); A;: LSMeans within the same row without a common superscript differ significantly (p < 0.05)
Discussion

Parenterally administrated bacterial LPS triggers dose-dependently acute phase response with subsequent systemic inflammation as part of the non-specific, innate immune defence in chicken (XIE et al. 2000; WANG et al. 2003; SHINI et al. 2008). The LPS recognition complex including Toll-like receptor-4 (TLR4) and co-receptors CD14 and MD-2 (POLTORAK et al. 1998; KOGUT et al. 2005; PARK et al. 2009) acts as crucial sentinel on the surface of antigen-presenting cells such as macrophages, dendritic cells and heterophils detecting LPS as host foreign (FARNELL et al. 2003; LEVEQUE et al. 2003; IQBAL et al. 2005). These cells produce reactive oxygen and nitrogen intermediates such as NO in a substrate-limited reaction by Arg and release pro-inflammatory cytokines similar to mammalian IL-1β, IL-6 and TNF-α (KLASING et al. 1987; KLASING and PENG 1990; KLASING and JOHNSTONE 1991; STAEHELI et al. 2001). Latter ones are of pivotal importance for coordinating acute phase reaction characterised by fever response, sickness behaviour and anorexia through IL-1β activity (MACARI et al. 1993; KLASING 1994; XIE et al. 2000), hepatic synthesis and secretion of acute phase proteins through IL-6 activity (KLASING and JOHNSTONE 1991; GRUYS et al. 2005) as well as protein breakdown and muscle wasting through TNF-α activity (COONEY et al. 1997; GRUYS et al. 2005). Based on these mechanisms the present study aimed to examine immunonutritive effects of a long-term graded Arg supply on haematological and febrile response in 12-week-old cockerels of different genotypes suffering systemic inflammation.

In the present study the injected LPS induced an acute phase response with severe alterations in cockerels’ DFI and DWG, body temperature as well as differential blood counts. In accordance to XIE et al. (2000), CHENG et al. (2004) and TAN et al. (2014) a marked anorexia with subsequent body weight loss characterised the unspecific sickness behaviour of present cockerels mainly. KLASING and JOHNSTONE (1991) and KLASING and KORVER (1997) have pointed out that sickness-induced anorexia lead to growth depression and decreased production efficiency in poultry further. The strong correlation between feed intake and weight gain found in the present study indicated that the loss of body weight could result from two processes: Firstly, anorexia and subsequent fasting cause ingesta emptying of gastrointestinal tract per se. Secondly, the activity of pro-inflammatory cytokine TNF-α intensifies the sickness-induced anorexia by initiating muscular protein breakdown in order to
mobilise energy and amino acids required for fever response and hepatic synthesis of acute phase proteins (BARACOS et al. 1987; CHIOLÉRO et al. 1997; COONEY et al. 1997; GRUYS et al. 2005). Because the amino acid pattern required in these particular metabolic pathways differs from that provided from protein breakdown, increased nitrogen excretion with following decreased nitrogen retention and growth retardation are described in rats and pigs suffering from acute-phase response (DICKERSON et al. 1997; BREUILLE et al. 1999; DICKERSON et al. 2001; BRUINS et al. 2002). Therefore, nitrogen balance studies are of fundamental significance in examining sickness-induced catabolism of the skeletal musculature and anabolism of the immune system (KONSTANTINIDES 1992).

In addition to the beneficial effects of dietary Arg on avian immune function (KWAK et al. 2001; DENG et al. 2005; TAYADE et al. 2006; MUNIR et al. 2009; TAN et al. 2014), the present study revealed an intensification of LPS-induced body weight loss in deficiently Arg supplied cockerels. Because chickens fed with deficient dietary Arg have a smaller pool of available plasma Arg (CHU and NESHEIM 1979; KWAK et al. 1999), sickness-induced anorexia could exacerbate low plasma Arg further, as known from pigs and humans (BRUINS et al. 2002; SUCHNER et al. 2002), and elevates chickens’ Arg requirement under pathophysiological conditions (KWAK et al. 2001). Furthermore, experiments on acute injured rats describe protein metabolism improving effects of supplemented Arg by limiting protein and body weight loss during acute inflammatory response (BRAGA et al. 1999; CUI et al. 1999). The actual mechanisms underlying the beneficial properties of adequate and surplus Arg are not completely investigated, but the following properties of Arg could be considered: Firstly, chickens are unable to synthesise Arg de novo (TAMIR and RATNER 1963) and require larger amounts of Arg in pathophysiological processes with subsequent anorexia (KWAK et al. 2001). Secondly, Arg contains the largest nitrogen proportion of proteinogenic amino acids that could spare muscular protein breakdown and improve nitrogen balance subsequently (BRAGA et al. 1999; CUI et al. 1999). Additionally, Arg serves as precursor of growth-promoting polyamines and muscle creatine that could enhance nitrogen retention further (KHAJALI and WIDEMAN 2010). Finally, Arg has secretagogue activities stimulating the release of pancreatic (e.g. glucagon, insulin, insulin-like growth factor-1; DORSHKIND and HORSEMAN 2000; CALDER and YAQOOB 2004) and pituitary
hormones (e.g. growth hormone, prolactin; BARBUL 1986; SCANES 2009) that influence protein, fat and carbohydrate metabolism as well as immunological processes strongly. Moreover, genotype-dependent differences in DWG and DFI were also seen in the present study. Based on their genetically determined higher body weight, brown genotypes showed higher DWG than lighter weighted white genotypes (LIEBOLDT et al. 2015 a), but brown BLA even achieved lowest DFI surprisingly. However, these genotype-dependent differences in DFI disappeared during the first 24 hours p. inj., since LPS induced equally low feed intake. That leads to the assumption that severe sickness-induced anorexia might cover potential genotype-dependent differences in feed intake. Depending on the strong correlation between DFI and DWG, an equally low feed intake would result in varying DWG responses in physiologically divergent genotypes. As a result, the present study revealed that the subsequent loss of body weight responded genotype-specific to preceding anorexia, because low performing cockerels lost body weight to a lesser extent than high performing ones in the first 24 hours p. inj.. In the following, brown genotypes and high performing white WLA compensated body weight loss more efficiently than low performing white R11 depending on their greater feeding capacity.

In addition, measureable changes in cockerels’ body temperature mediated by pro-inflammatory cytokines accompanied the severe alterations in DWG and DFI during acute phase response. Each genotype exhibited a unique pattern of fever response and recovery from LPS-induced immunological stress. In brown genotypes LPS induced an initial hypothermia followed by a slight hyperthermia. On the contrary, white genotypes showed biphasic hyperthermia only. Similar to the present results, previous studies have demonstrated that LPS initiates fever in chicks differently, such as biphasic response divided in an initial phase of hypothermia followed by hyperthermia (ROTIROTI et al. 1981; DE BOEVER et al. 2008) or divided in a biphasic hyperthermia (KOH et al. 1996; XIE et al. 2000). Such differences in avian fever response found between studies may result from differences in used aetiological pathogen, serotype, batch or dosage of LPS, its route of application, host’s sensitivity to LPS as well as the magnitude and duration of the induced inflammation. However, the found fever response differences between genotypes lead to the assumption that the genetic background of chicken may be a potential cause for contrasts in avian capability to resist stress. CLARK et al. (2009) have emphasised that beside pathogen associated reasons
the environment, genetic background, age, sex, hormonal, nutritional and health status as well as individual characteristics of chickens have to be taken into account for the interpretation and comparison of immunological studies. Variations in LPS sensitivity and its associated resistance to diseases are described for different chicken lines and strains that may result from variations in TLR expression (ABASHT et al. 2009) and gene polymorphisms of varying TLR number on avian macrophages (DIL and QURESHI 2002 b; LEVEQUE 2003) between genotypes. The subsequent differences in the amount of released pro-inflammatory cytokines and reactive intermediates may be responsible for the in magnitude and duration varying immune responses of the genotypes in the present study.

Moreover, the alterations described above were associated with significant absolute and relative changes in peripheral WBC from physiological to pathophysiological conditions. As physiological haemoresponce depends on a large variety of influencing factors from environment and management practices (FOURIE and HATTINGH 1980; WANG et al. 2003; CLARK et al. 2009), comparisons of haematological studies in birds are characterised by large variation. Mediating acute inflammatory response to bacteria the first line of cellular defence comprised phagocytic cells such as heterophils (reviewed in HARMON 1998), thrombocytes (CARLSON et al. 1968; ST. PAUL et al. 2012) and monocytes (GRECCHI et al. 1980; STABLER et al. 1994). These cells bind, phagocytise and trigger killing of bacteria via antimicrobial substances such as superoxides in chicken and turkey heterophils (STABLER et al. 1994) and NO in chicken monocytes (BOWEN et al. 2007, 2009) and thrombocytes (FERDOUS et al. 2008; SCOTT and OWENS 2008; ST. PAUL et al. 2012). Through the release of chemotactic molecules phagocytes attract lymphocytes (QURESHI 2003), the second line of defence important for both cellular and humoral mediated immunity in late inflammatory response. With reference to the findings of XIE et al. (2000) and WANG et al. (2003) in commercial broilers, the cockerels of the present experiment showed a significant leukopenia within 4 hours p. inj. due to lymphopenia as well as basophil and eosinophil granulocytopenia. In agreement with LATIMER et al. (1988) and HARMON (1998) an early increase of circulating heterophils indicates acute inflammation from 8 to 24 hours p. inj. due to their release from bone marrow and their movement from the marginal pool. This time-dependent reciprocal relationship between heterophils and lymphocytes proportions is frequently seen in immunologically stressed chickens (SCANES 2015) and
verified in the present study. These changes base on the different response of heterophils and lymphocytes to stress (DAVISON et al. 1983; GROSS and SIEGEL 1983). The authors have reported on severe heterophilia associated with a strong lymphopenia due to corticosterone administration in young chicken and have deduced that the H/L ratio serves as reliable and accurate physiological indicator for avian stress response. For classification of experimental results H/L ratios equal or higher than 0.8 are defined as high-degreed stress in chicken (GROSS and SIEGEL 1983). In response to LPS, XIE et al. (2000) and WANG et al. (2003) have found H/L ratios higher than 1.0 at 8 to 12 hours p. inj. in chicken that decrease afterwards. These findings were in agreement with the present results, in which H/L ratios take values over 1.0 from 4 to 8 hours p. inj. and decreased strongly afterwards. Consequently, the acute phase response has to be considered as the most stressful period lasting for 8 to 12 hours p. inj.. Coordinated by the effects of pro-inflammatory cytokines immunological stress seemed to be metabolically intensified by the anorexia and the subsequent change from anabolism to catabolism during acute inflammation. However, the H/L ratio returned to pre-injection values and provided haematological indication for the subsidence of stressful conditions during cockerels’ recovery in DFI and DWG in the second 24 hours p. inj..

Although cockerels recovered visible from systemic inflammation in feed intake and fever response until 48 hours p. inj., the peripheral WBC revealed severe leukocytosis, thrombocytosis and monocytois further. These findings are in accordance to the results of LATIMER et al. (1988), XIE et al. (2000) and BOWEN et al. (2007) and can be considered as evidence for the importance of avian thrombocytes and monocytes in the delayed immune response with transition to chronic processes as well as for their regeneration from bone marrow to compensate their previous wasting. In both thrombocytes and monocytes LPS even stimulates the iNOS expression and hence NO production (BOWEN et al. 2007, 2009; ST. PAUL et al. 2012). Because white genotypes show a significantly larger proportion of monocytes at 24 hours p. inj. than brown genotypes and BLA had a smaller proportion of thrombocytes at 48 hours p. inj. than the other genotypes, these NO producing cells may be responsible for the different outcome of immune response in genotypes in the present study. Although the strong effects of LPS on the avian immune response predominated in the present study, the insufficient Arg supply of 70 % of recommended level (NRC 1994) from hatch to
PAPER IV

12th week of age caused higher total leukocytes counts than the 100 % and 200 % Arg diets. Even the H/L ratio tended to be influenced by imbalanced graded Arg supply of 70 % and 200 % of recommended Arg level. With reference to DAVISON et al. (1983) and GROSS and SIEGEL (1983) considering H/L ratios higher than 0.8 as high-degree stress, it can be assumed that dietary imbalances of Arg intensify the immunological stress in sick cockerels on a metabolic level additionally. But indications for an Arg derived attenuation of pathophysiological activities induced by overexpressed pro-inflammatory cytokines as well as a stimulation of cell-mediated systemic immune response in immune challenged chickens as described by TAYADE et al. (2006), MUNIR et al. (2009) and TAN et al. (2014) were not found in the present examination of cockerels.

In conclusion, the present results confirmed that LPS acts as a potent immune inducer causing numerous and diverse immunological changes in 12-week-old cockerels. The study emphasized that chicken genotype obviously is a source of variation to be considered in immunological studies. Although benefits of supplementing Arg far beyond the NRC recommendation are not found in the present study, insufficient dietary Arg serves as additional stressor to LPS-induced body weight loss and alterations in differential blood count. For that reason, an adequate Arg supply has to be ensured in diets of growing chickens.

Acknowledgements

The authors gratefully acknowledge Mrs. Annerose Junghans for the care of the experimental animals and Ms. Lara Lindner for the preparation and staining of the blood smears.

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Metabolic and Clinical Response to *Escherichia coli* Lipopolysaccharide in Purebred Layer Pullets of Different Genetic Backgrounds Supplied with Graded Dietary L-arginine

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**Poultry Science**

Submitted
Abstract
L-arginine (Arg) is an essential amino acid in birds that plays a decisive role in avian protein synthesis and immune response. Effects of graded dietary Arg supply on metabolic and clinical response to *Escherichia coli* lipopolysaccharide (LPS) were studied over 48 hours after a single intramuscular LPS injection in 18-week-old genetically diverse purebred pullets. LPS induced a genotype-specific fever response within 4 hours post injectionem. Whereas brown genotypes showed an initial hypothermia followed by longer-lasting moderate hyperthermia, white genotypes exhibited a biphasic hyperthermia without initial hypothermia. Furthermore, within 2 hours after LPS injection sickness behavior characterized by lethargy, anorexia, intensified respiration and ruffled feathers appeared, persisted for 3 to 5 hours and recovered until 12 hours post injectionem. The varying grades of Arg did not alter the examined traits named above, whereas insufficient Arg reduced body growth and increased relative weights of liver and pancreas significantly. At 48 hours post injectionem increased relative weights of liver and spleen were also found in LPS treated pullets, whereas LPS decreased those of pancreas, bursa, thymus and cecal tonsils. Moreover, LPS lowered the sum of plasma amino acids and decreased plasma concentrations of Arg, citrulline, glutamate, methionine, ornithine, phenylalanine, proline, tryptophan and tyrosine, and increased those of aspartate, glutamine, lysine, 1- and 3-methyl-histidine. Elevating concentrations of dietary Arg led to increasing plasma concentrations of Arg, citrulline, ornithine and 3-methyl-histidine subsequently. As quantitative expression of lipopolysaccharide-induced anorexia, proteolysis and the following changes in plasma amino acids, pullets showed a significant decrease of feed and nitrogen intake and catabolic metabolism characterized by negative nitrogen balance and body weight loss in the first 24 hours post injectionem. Pullets recovered from the challenge within the second 24 hours post injectionem and changed to anabolism with re-increased feed and nitrogen intake, positive nitrogen retention and weight gain. To conclude, present results confirmed that LPS induced numerous metabolic and physiological changes in pullet’s genotypes, whereas dietary Arg affected the examined traits only slightly.

**Key words:** arginine, clinical response, *Escherichia coli* lipopolysaccharide, metabolic response, pullet
Introduction

Modern poultry industry is continuously facing rapid changes such as the aspired reduction of prophylactic and therapeutic antibiotic’s usage. Because this development can increase the impact of stressors on bird health and welfare (BARNETT and HEMSWORTH 2003), dietary bioactive components influencing poultry’s immunocompetence such as L-arginine (Arg) are more in the focus during the past years (KOUTSOS and KLASING 2001; HUMPHREY and KLASING 2004; TAN et al. 2014). Due to birds’ lack of urea cycle key enzymes chickens are unable to synthesize Arg de novo from L-ornithine and depend completely on dietary Arg to meet their needs for protein synthesis and other biological functions (TAMIR and RATNER 1963). CHU and NESHEIM (1979) and KWAK et al. (1999 and 2001) have confirmed that chicken’s plasma Arg is directly influenced by dietary intake and catabolic processes such as protein breakdown. In addition, DIETERT et al. (1994) have suggested that the conversion of Arg to nitric oxides (NO) during inflammatory response can reduce Arg availability for other metabolic pathways. In this context, BRUINS et al. (2002) have emphasized that plasma Arg concentrations are decreased in pigs suffering from endotoxemia. This situation seems to be applicable to birds, as chickens show an immunological Arg requirement that differs between healthy and immune-challenged birds (KWAK et al. 2001; HUMPHREY and KLASING 2005). In the immune system Arg plays a decisive role as only known precursor of NO, synthesized by induced nitric oxide synthase (iNOS). NO is a paracrine immune mediator and a cytotoxic product of activated avian macrophages (QUreshi 2003) whose production is substrate-limited by Arg (WIDEMAN et al. 1995; KIDD et al. 2001; RUIZ-FERIA et al. 2001). The immune modulating properties of Arg in chicken have also been demonstrated by the results of KWAK et al. (1999), LEE et al. (2002) and TAN et al. (2014), as dietary Arg level modulates lymphoid organ development, alters percentages of leukocytes in peripheral blood after challenging with pathogens and changes proportions of T cell subpopulations. However, DENG et al. (2005) have emphasized that Arg supplementation has minimal effects on short-term immunological responses, but enhances longer-term antibody responses after pretreatment with supplemental Arg.

Lipopolysaccharide (LPS) from cell wall of Gram-negative bacteria is frequently used to induce experimental acute-phase reaction associated with a systemic inflammation (Xie et al. 2000; LESHCHINSKY and KLASING 2001; CHENG et al. 2004), which is characterized by
the release of NO and the avian equivalents of pro-inflammatory cytokines IL-1β and IL-6 from avian leukocytes (KLASING et al. 1987; DIL and QUreshi 2002 a; FARNELL et al. 2003). Although tumor-necrosis-factor (TNF)-like activities are observed in chickens, an avian orthologue of mammalian pro-inflammatory TNF-α and its associated encoding gene sequence have not been found in birds to date (KAISER et al. 2005; KAISER and STAHELI 2014). The released pro-inflammatory mediators cause fever, hepatic secretion of acute-phase proteins, and sickness behavior such as anorexia and lethargy (XIE et al. 2000; LESHCHINSKY and KLASING 2001; CHENG et al. 2004). Pro-inflammatory cytokines simultaneously induce significant changes in protein metabolism by skeletal muscle protein breakdown and muscle wasting (ROSENBLATT et al. 1983; SAX et al. 1988). Amino acids derived from protein catabolism and dietary intake are used for hepatic gluconeogenesis and acute-phase protein synthesis as well as by further tissues and cells involved in inflammatory and immune response (KLASING and AUSTIC 1984; KLASING 1988; BARNES et al. 2002). Because the amino acid pattern required in these particular metabolic pathways is different from that derived from skeletal muscle proteolysis, protein loss, growth retardation, decreased nitrogen (N) retention, and increased N excretion occur in humans and animals suffering from acute-phase reaction (VOisin et al. 1996; BREUILLE et al. 1999, BRUINS et al. 2002).

Since VAN EERDEN et al. (2004) have found differences in antibody response between low and highly efficient pullets and KWAK et al. (2001) have reported on genetically dependent differences in Arg requirement and immune response of different Cornell K strains, we hypothesized that chicken strains capable of producing different amounts of egg mass each day may present different immunological and metabolic responses to LPS-induced acute-phase reaction under varying feeding conditions. Therefore we developed further an animal model consisting of four purebred layer lines (LIEBOLDT et al. 2015 a) in order to get a deeper insight in the interaction of avian genetic background and dietary environment, in regard to their metabolism and immune response. As layer-type birds are exposed to various stressors influencing growth, metabolism and immunity during rearing, our objective was to investigate the metabolic and clinical response of 18-wk-old pullets of four purebred layer lines adapted to three different levels of dietary Arg and one-time-treated with an intramuscular injection of E.coli LPS.
Material and Methods

Birds, husbandry, experimental design and diets

The experiment (Figure 1) was conducted with eighteen 18-wk-old pullets of four purebred layer lines each. Two commercial high performing genotypes (WLA and BLA) with average 50 g egg mass/d per hen over 364 days were contrasted to two low performing ones (R11 and L68) with average 26 to 30 g egg mass/d per hen over 364 days. The high performing lines were taken from a layer breeding program of Lohmann Tierzucht GmbH, Cuxhaven, Germany, and the low performing ones derived from non-selected resource populations at 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 the Rhode Island Red line BLA and its counterpart L68 (New Hampshire). Birds were randomly selected from a rearing trial carried out simultaneously (LIEBOLDT et al. 2015 b), in which chicks were fed from hatch to laying period with graded concentrations of dietary Arg equivalent to 70, 100 and 200 % of age-specific recommended supply (NRC 1994). After hatch birds were equipped with a wing-tag and vaccinated against Marek’s and Newcastle Disease.

For the present study birds were housed in metabolic single-cages (42 cm x 35 cm x 42 cm) of a three-floor battery equipped with outside feed trough and water bowl in an environmentally controlled room. During the experiment temperature was between 18 to 19 °C and light period lasted from 0500 to 1600 h (11L:13D). Feed and water were provided ad libitum. The 18 pullets of each genotype were distributed to 3 experimental diets with 6 replicate cages. Experimental diets contained dietary Arg equivalent to 70, 100 and 200 % Arg of age-specific recommended supply (Table 1; NRC 1994). The diets for pullets comprised a basal diet with no further Arg supplementation (low Arg, LA; 4.74 g Arg/kg diet), the basal diet with adequate Arg (AA; 6.46 g Arg/kg diet) and high Arg supplementation (HA; 13.44 g Arg/kg diet). The basal diet was supplemented with any deficient essential amino acid other than Arg. For AA and HA, Arg (free base, 99 %, Europepta, Hannover, Germany) was added to the basal diet at the expense of corn.

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.
Figure 1. Time course of the experiment subdivided into periods of adaptation, recovery from data logger implantation into body cavity and the following nitrogen (N) balance trial (a) as well as the experimental design with distribution of experimental groups (b). Pullets of four genotypes were supplied with low (LA), adequate (AA) and high (HA) dietary L-arginine. After 5 days of the N balance trial pullets were treated with 2 mg \textit{E.coli} lipopolysaccharide (LPS) per kg BW or 0.9 % saline solution (NaCl) intramuscularly. The experiment ended with the slaughtering of the pullets.
Table 1. Ingredient composition, analysis and calculation (g/kg diet) of low (LA), adequate (AA) and high (HA) L-arginine supplied experimental diets.

<table>
<thead>
<tr>
<th>Ingredients (g/kg diet)</th>
<th>LA</th>
<th>AA</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>300.0</td>
<td>300.0</td>
<td>300.0</td>
</tr>
<tr>
<td>Wheat</td>
<td>150.0</td>
<td>150.0</td>
<td>150.0</td>
</tr>
<tr>
<td>Triticale</td>
<td>147.5</td>
<td>147.5</td>
<td>147.5</td>
</tr>
<tr>
<td>Corn</td>
<td>209.5</td>
<td>208.0</td>
<td>201.5</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>80.0</td>
<td>80.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Lucerne pellets</td>
<td>60.0</td>
<td>60.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Vitamin-trace mineral premix&lt;sup&gt;1&lt;/sup&gt;</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>L-lysine HCl</td>
<td>2.6</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>L-arginine</td>
<td>-</td>
<td>1.5</td>
<td>8.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
</tr>
</tbody>
</table>

**Analysed values (g/kg diet)**

<table>
<thead>
<tr>
<th></th>
<th>LA (g/kg)</th>
<th>AA (g/kg)</th>
<th>HA (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>888.4</td>
<td>891.4</td>
<td>891.7</td>
</tr>
<tr>
<td>Crude ash</td>
<td>52.1</td>
<td>53.2</td>
<td>54.2</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>20.5</td>
<td>21.9</td>
<td>23.1</td>
</tr>
<tr>
<td>Crude fat</td>
<td>30.7</td>
<td>31.6</td>
<td>34.6</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>38.5</td>
<td>39.3</td>
<td>39.3</td>
</tr>
<tr>
<td>Starch</td>
<td>493.2</td>
<td>491.8</td>
<td>482.8</td>
</tr>
<tr>
<td>Sucrose</td>
<td>23.8</td>
<td>24.3</td>
<td>23.0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>13.9</td>
<td>14.3</td>
<td>13.8</td>
</tr>
<tr>
<td>Calcium</td>
<td>11.5</td>
<td>11.8</td>
<td>11.6</td>
</tr>
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</table>

**Calculated values (MJ/kg or g/kg diet)**

<table>
<thead>
<tr>
<th></th>
<th>LA (g/kg)</th>
<th>AA (g/kg)</th>
<th>HA (g/kg)</th>
</tr>
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<tbody>
<tr>
<td>AMEN&lt;sup&gt;2&lt;/sup&gt;</td>
<td>11.6</td>
<td>11.7</td>
<td>11.8</td>
</tr>
<tr>
<td>Methionine&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.43</td>
<td>2.43</td>
<td>2.42</td>
</tr>
<tr>
<td>Lysine&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6.26</td>
<td>6.26</td>
<td>6.24</td>
</tr>
<tr>
<td>Arginine&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.74</td>
<td>6.46</td>
<td>13.44</td>
</tr>
</tbody>
</table>

<sup>1</sup>Premix (Deutsche Vilomix Tierernährung GmbH, Neuenkirchen-Vörden, Germany) contained per kg of diet: vitamin A, 10,000 IU (retinyl acetate); vitamin D<sub>3</sub>, 2,000 IU (cholecalciferol); vitamin E, 25 IU (DL-α-tocopheryl-acetate); vitamin B<sub>1</sub>, 2.5 mg; vitamin B<sub>2</sub>, 5.0 mg; vitamin B<sub>6</sub>, 4.0 mg; vitamin B<sub>12</sub>, 18.5 µg; vitamin K<sub>3</sub>, 3.0 mg; nicotinic acid, 30 mg; pantothenic acid, 9.0 mg; folic acid, 0.8 mg; biotin, 21 µg; choline chloride, 300 mg; iron from ferrous-(II)-sulphate monohydrate, 40 mg; copper from cupric-sulphate pentahydrate, 15 mg; manganese from manganese-(II)-oxide, 80 mg; zinc from zinc-oxide, 80 mg; iodine from calcium iodate, 1.6 mg; selenium from sodium selenite, 0.3 mg; cobalt from basic cobalt-(II)-carbonate monohydrate, 0.2 mg; Butylated hydroxy toluene, 100 mg.

<sup>2</sup>Calculation according to energy estimation equation of the World’s Poultry Science Association (VOGT 1986).

<sup>3</sup>Calculation based on analyzed amino acid contents of ingredients and their proportions of the diets.

Core body temperature measurements

In the 16<sup>th</sup> wk of age, after 7 days of adaptation to environmental conditions, pullets underwent surgery in order to insert a temperature data logger (DS1921H-F5 Thermochron iButton High Resolutions, Maxim Integrated, San Jose, CA) with a size of 17.35 x 5.89 mm and a weight of 3.3 g into body cavity. Temperatures were measured from +15 to +46 °C with a resolution of 0.125 °C. Data loggers were calibrated and programmed to continuously record of core body temperature (CBT) every 10 min over the 7 days lasting experiment. Before implantation data loggers were encapsulated in a sterile plastic cover (ring cap,
EndoTherm GmbH, Arlesheim, Switzerland). In order to minimize failures in anesthesia, pullets were fasted overnight and water was restricted one hour before surgery. To enable preoperative analgesia, butorphanol (2.5 mg/kg BW, Alvegesic vet., CP Pharma, Burgdorf, Germany) was injected into left pectoral muscle. General anesthetic was induced by xylazine (1.5 mg/kg BW, Xylazin 2%, Albrecht GmbH, Aulendorf, Germany) and ketamine (3.0 mg/kg BW, Ketamin 100 mg/ml, CP Pharma, Burgdorf, Germany) injected into right pectoral muscle. After loss of consciousness pullets were anaesthetized further with 2% isoflurane inhalation (Isofluran CP 1 ml/ml, CP Pharma, Burgdorf, Germany) in oxygen via a head chamber. Once pullets were fully anaesthetized, abdominal feathers were plucked, operating field was cleaned, degreased and sterilized and body cavity was carefully opened by 4 cm longitudinal incision in the ventral abdominal wall behind sternum. Encapsulated data loggers were carefully inserted into body cavity. Thereafter, the surgical wound was closed stepwise with absorbable suture (4-0 USP, Vicryl, Johnson & Johnson Medical GmbH, Norderstedt, Germany). Before cutaneous wound was sutured 1 ml benzylpenicillin (Veracin compositum, Albrecht GmbH, Aulendorf, Germany) was given on the muscle suture in order to avoid bacterial wound infection. To allow good recovery, pullets were put into a box with heat lamp until consciousness and standing regained completely. Afterwards pullets were placed back into their metabolic cages. As part of a multimodal pain management butorphanol (2 mg/kg BW) was administrated 12 hours post-surgery intramuscularly. During 10 days of recovery, pullets remained healthy and had no signs of adverse implications from surgery that could affect experimental procedure.

Experimental procedure

The actual experiment lasted 7 days and was performed at the 18th wk of age (Figure 1). Pullets had diverse initial BW, as they originated from different genotypes reared with the three diets used in this study further (LIEBOLDT et al. 2015b). There was no BW difference between WLA (1040 g) and BLA (1070 g), but R11 (880 g) and L68 (1230 g) showed significant differences. Pullets reared with insufficient dietary Arg weighed about 60 g less than AA and HA fed birds at the beginning of the experiment. The present trial was subdivided into two parts: examination of physiological conditions from days 1 to 5 and LPS-induced pathophysiological conditions at days 6 and 7. After recording pullets’ BW the
experiment began at 0700 h on day 1. In order to calculate N retention of pullets under physiological and pathophysiological conditions, feed intake was recorded and total excrements were collected individually. During the entire trial residual feed was recorded daily and pullets’ excrements were collected twice a day at 0700 h and 1500 h. Excrements from days 1 to 5 were pooled for each pullet individually, but those at day 6 and 7 were separated for each pullet and day. After collection excrements were stored at -20 °C until further sample processing. On day 6 at 0700 h pullets’ BW was recorded again and three pullets of each experimental group (Figure 1) were challenged with 2 mg E.coli LPS /kg BW (serotype O111:B4, Sigma Aldrich Chemie GmbH, Munich, Germany), diluted in a sterile saline solution (0.9 % NaCl) to 2 mg LPS/ml, and the other half of each group was treated with 1 ml sterile saline solution (0.9 % NaCl) into the left pectoral muscle. Saline solution was chosen as control reagent, because it did not affect CBT or induce immunological changes. The BW of pullets was recorded at 24 and 48 hours post injectionem (p. inj.).

From one hour before treatment pullets were clinically monitored at 20 min intervals. As birds’ behavior and condition appeared normal by 12 hours p. inj., a further examination of clinical symptoms of these completely recovered pullets was omitted. To characterize sickness behavior, the clinical traits body posture, behavior, plumage and respiration were semi-quantitatively evaluated by a scoring system (Table 2) from physiological condition (score 0) to severe deviation from physiological status (score 4). As saline treated pullets showed no changes in clinical traits (score 0), these results were not graphically presented.

The experiment ended 48 hours p. inj. by slaughter of all pullets and blood sampling through exsanguination of neck vessels in 10 ml tubes containing lithium heparin (Sarstedt AG & Co, Nümbrecht, Germany). For analysis of plasma amino acids, blood samples were immediately centrifuged at 1500 x g and 4 °C for 15 min and plasma was divided into two aliquots and stored at -80 °C until analyzed. Plasma amino acid concentrations were determined by the HPLC method described by KUHLA et al. (2010).

After exsanguination implanted data loggers were retrieved from pullets’ body cavity and the data downloaded for analysis via an USB interface and the software Thermodata Viewer (Thermodata Corporation, Whitewater, WI). Simultaneously, weights of heart, liver, gizzard, pancreas, thymus, bursa of Fabricius, spleen and cecal tonsils were recorded and their weights were presented as relative weights of BW (% of BW = [organ weight/BW] x 100).
Table 2. Semi-quantitative scoring system for the evaluation of clinical symptoms.

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>Scoring point</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>physiological</td>
</tr>
<tr>
<td>Body posture</td>
<td>1</td>
<td>standing, huddled, head upright</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>standing, huddled, head lowered</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>lying, huddled, head lowered</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>lateral position</td>
</tr>
<tr>
<td>Behavior</td>
<td>0</td>
<td>physiological</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>reduced activity and attention</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>somnolent</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>apathetic</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>comatose</td>
</tr>
<tr>
<td>Plumage</td>
<td>0</td>
<td>physiological</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>slightly ruffled</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>moderately ruffled</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>extremely ruffled</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>feather loss (initiating alopecia)</td>
</tr>
<tr>
<td>Respiration</td>
<td>0</td>
<td>physiological</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>slightly enhanced</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>moderately enhanced (initiating abdominal respiration)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>extremely enhanced (significant abdominal respiration)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>dyspnoea (beak respiration)</td>
</tr>
</tbody>
</table>

Analysis of feed and excrements

The individual samples of pooled total excrements from days 1 to 5, and those of day 6 and day 7 were defrosted at room temperature (ca. 20 °C), homogenized and freeze-dried for 48 hours. Afterwards freeze-dried excrements were ground to pass a 0.5 mm screen and analyzed for DM and Dumas N. Experimental diets (Table 1) were analyzed for DM, Kjeldahl N, crude ash, crude fat, crude fiber, starch, sucrose, phosphorous and calcium. All analysis was in accordance to the methods of the Association of German Agricultural Analytic (VDLUFA; BASSLER 1997). The AME_N of diets was calculated according to the energy estimation equation of the World’s Poultry Science Association (VOGT 1986). In order to calculate the concentrations of amino acids in the experimental diets appropriately, amino acid containing feed components others than those supplemented in their free forms were analyzed for the amounts of containing amino acids by ion exchange chromatography described in the analytical methods of AMINODat 4.0 (EVONIK INDUSTRIES 2010).

Nitrogen balance

Based on the individually recorded BW and residual feed, daily weight gain (DWG) and daily feed intake (DFI) were calculated for each pullet under physiological (days 1 to 5) and pathophysiological conditions (day 6 and day 7). Furthermore, N intake was determined by
multiplying pullets’ feed intake by analyzed Kjeldahl N concentration of the corresponding diet. Based on total excrements (days 1 to 5 as well as day 6 and day 7) the N excretion was calculated by multiplying the amount of excreta by its analyzed Dumas N concentration. Finally, N retention was determined subtracting N excretion from N intake. To account for genotype-dependent BW differences in growth associated parameters and in N metabolism all measures of DWG, DFI and N balance (N intake, N excretion, and N retention) were raised to the power of 0.67, i.e. the metabolic BW (kg BW^{0.67}).

**Statistical data analysis**

Although semi-quantitative clinical symptoms were just recorded by a 5-degree score, these data were evaluated by the same procedures as the metric parameters to enable a complex time-dependent evaluation and to discuss the scores as group-specific least square means. CBT, measured every 10 min, was averaged for 30 min intervals per pullet and graphically presented as least square means for each 30 min interval.

Statistical evaluation was performed using the software package of SAS 9.4 (SAS INSTITUTE INC. 2012, Cary, NC). In general, procedure MIXED was used for evaluating the data. Fixed effects were “genotype” (WLA, BLA, R11 and L68), “diet” (LA, AA and HA), “treatment” (LPS or saline), “time” (observation specific time levels) and their interactions. As relative organ weights and absolute plasma amino acid concentrations were only evaluated at 48 hours p. inj., fixed effect “time” was excluded from evaluation of these traits. The model was formulated to account for heterogeneity of variances and degrees of freedom were estimated using the “kr” statement. Co-variance structure was modeled by a *compound symmetry* time-dependent repeated structure within pullets. As traits were measured repeatedly on the same pullet, a “repeated” statement was considered in the statistical model to account for similarities within subjects. The described model and covariance structure were found to be most appropriate according to the AICC.

Effects were considered to be significant at a probability level lower or equal to 0.05. The Tukey-Kramer test was applied for a multiple comparison of means. Based on the described model the mean value differences were evaluated and presented in two different ways using the “pdiff” statement. First, for each trait the difference between the initial value before treatment and for a particular time was tested by *t*-test within each treatment. Secondly,
treatment differences were evaluated separately for each time. Least square means and standard errors were presented graphically along with the statistics.

**Results**

*Daily weight gain, nitrogen intake and retention*

DWG (Figure 2), DFI (Table 3), daily N intake (Figure 3) and daily N retention (Figure 4) were affected by “genotype”, “treatment” and “time” as well as the interactions of “genotype and time” and “treatment and time” \( (p < 0.05) \). An Arg effect on these four traits was not found (DWG: \( p_{Diet} = 0.836 \); DFI: \( p_{Diet} = 0.742 \); N intake: \( p_{Diet} = 0.336 \); N retention: \( p_{Diet} = 0.524 \)). Brown genotypes showed higher DWG, DFI and daily N intake than white ones during the entire trial period \( (p_{Genotype} < 0.001; p_{Genotype*Time} < 0.001) \). Additionally, L68 showed a higher daily N retention than the other genotypes \( (p_{Genotype} < 0.001) \), which did not differ from each other. Saline treated pullets had a higher weight gain, feed intake, N intake and N retention than those treated with LPS \( (p_{Treatment} < 0.001) \). However, genotype-specific DWG, DFI, daily N intake and N retention did not differ between LPS and saline treated pullets before treatment, whereas all four traits decreased within the first 24 hours \( p. \ inj. \) and increased within the second 24 hours \( p. \ inj. \) in both treatment groups \( (p_{time} < 0.001) \). Feed intake and subsequent N intake decreased to 10 to 20 % of baseline and control group values in LPS treated white pullets and to 20 to 30 % of the baseline and control group values in LPS treated brown pullets \( (p < 0.001) \). Saline treated R11 pullets also showed a strong loss of BW, but the other saline treated genotypes had only a reduced, but positive DWG \( (p_{Genotype*Treatment} < 0.001) \). However, BW of LPS treated pullets decreased to a greater extent than in saline treated pullets and even lost BW during the first 24 hours \( p. \ inj. \) \( (p_{Treatment*Time} < 0.001) \), whereas the lost BW did not differ between the genotypes. In saline treated pullets DWG, DFI, N intake and N retention regained initial values with the exception of DFI and N intake in the second 24 hours \( p. \ inj. \) of L68 \( (p_{Genotype*Treatment*Time} < 0.05) \). Traits also increased in LPS treated pullets but regained approximately 75 % of their baseline values within the same time period only \( (p_{Treatment*Time} < 0.001) \).
Table 3. Daily feed intake (g/kg$^{0.67}$ • d) during 5 days of physiological conditions *ante injectionem* (a. inj.) and 2 days *post injectionem* (p. inj.) in phylogenetic diverse pullets each selected for high (white WLA and brown BLA) and low (white R11 and brown L68) daily egg mass production, supplied with low (LA), adequate (AA) and high (HA) dietary L-arginine and treated with 2 mg *E. coli* lipopolysaccharide (LPS) per kg BW or 0.9 % saline solution (NaCl; LSMeans ± SE; n = 3).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Time</th>
<th>NA (p &lt; 0.001; pDiet = 0.742; pTreatment &lt; 0.001; pTime &lt; 0.001; pGenotype<em>Diet = 0.545; pGenotype</em>Treatment = 0.565; pGenotype<em>Time = 0.325; pDiet</em>Treatment = 0.248; pDiet<em>Time = 0.310; pTreatment</em>Time &lt; 0.001.)</th>
<th>AA</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NaCl</td>
<td>LPS</td>
<td>NaCl</td>
</tr>
<tr>
<td>WLA</td>
<td>a.inj. (D 1-5)</td>
<td>58.3bc</td>
<td>61.5bc</td>
<td>62.2bc</td>
</tr>
<tr>
<td></td>
<td>24 h p.inj. (D 6)</td>
<td>54.1bc</td>
<td>5.0d</td>
<td>62.5bc</td>
</tr>
<tr>
<td></td>
<td>48 h p.inj. (D 7)</td>
<td>61.3bc</td>
<td>37.7c</td>
<td>67.4bc</td>
</tr>
<tr>
<td>BLA</td>
<td>a.inj. (D 1-5)</td>
<td>79.6ab</td>
<td>64.2bc</td>
<td>67.4bc</td>
</tr>
<tr>
<td></td>
<td>24 h p.inj. (D 6)</td>
<td>65.0bc</td>
<td>11.5bc</td>
<td>49.3c</td>
</tr>
<tr>
<td></td>
<td>48 h p.inj. (D 7)</td>
<td>87.3bc</td>
<td>47.6c</td>
<td>86.7ab</td>
</tr>
<tr>
<td>R11</td>
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<td>66.0bc</td>
<td>50.0bc</td>
</tr>
<tr>
<td></td>
<td>24 h p.inj. (D 6)</td>
<td>61.0bc</td>
<td>10.2d</td>
<td>41.7bc</td>
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<tr>
<td></td>
<td>48 h p.inj. (D 7)</td>
<td>61.4bc</td>
<td>39.4c</td>
<td>45.5c</td>
</tr>
<tr>
<td>L68</td>
<td>a.inj. (D 1-5)</td>
<td>86.1bc</td>
<td>88.0bc</td>
<td>76.9ab</td>
</tr>
<tr>
<td></td>
<td>24 h p.inj. (D 6)</td>
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<td>19.6d</td>
<td>72.7b</td>
</tr>
<tr>
<td></td>
<td>48 h p.inj. (D 7)</td>
<td>71.6bc</td>
<td>54.6bc</td>
<td>69.9bc</td>
</tr>
<tr>
<td>PSEM</td>
<td>8.0</td>
<td>8.0</td>
<td>6.0</td>
<td>6.0</td>
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</table>
Figure 2. Daily weight gain (g/kg\(^{0.67}\) • d) during 5 days of physiological conditions ante injectionem (a. inj.) and 2 days post injectionem (p. inj.) in phylogenetic diverse pullets each selected for high [white WLA, a) and brown BLA, b)] and low [white R11, c) and brown L68, d)] daily egg mass production, supplied with low (LA), adequate (AA) and high (HA) dietary L-arginine and treated with 2 mg *E. coli* lipopolysaccharide (LPS) per kg BW or 0.9 % saline solution (NaCl; LSMeans ± SE; n = 3). ANOVA (p values): \(P_{\text{Genotype}} < 0.001; P_{\text{Diet}} = 0.836; P_{\text{Treatment}} < 0.001; P_{\text{Time}} < 0.001; P_{\text{Genotype*Diet}} = 0.495; P_{\text{Genotype*Treatment}} = 0.486; P_{\text{Genotype*Time}} < 0.001; P_{\text{Diet*Treatment}} = 0.284; P_{\text{Diet*Time}} = 0.505; P_{\text{Treatment*Time}} < 0.001. \) *; Bars within a diagram lacking a common superscript differ (\(p < 0.05\)).
Figure 3. Daily nitrogen (N) intake (mg N/kg<sup>0.67</sup> • d) during 5 days of physiological conditions ante injectionem (a. inj.) and 2 days post injectionem (p. inj.) in phylogenetic diverse pullets each selected for high [white WLA, a) and brown BLA, b) and low [white R11, c) and brown L68, d)] daily egg mass production, supplied with low (LA), adequate (AA) and high (HA) dietary L-arginine and treated with 2 mg E.coli lipopolysaccharide (LPS) per kg BW or 0.9 % saline solution (NaCl; LSMeans ± SE; n = 3). ANOVA (p values): p<sub>Genotype</sub> < 0.001; p<sub>Diet</sub> = 0.334; p<sub>Treatment</sub> < 0.001; p<sub>Time</sub> < 0.001; p<sub>Genotype*Diet</sub> = 0.921; p<sub>Genotype*Treatment</sub> = 0.432; p<sub>Genotype*Time</sub> < 0.05; p<sub>Diet*Treatment</sub> = 0.653; p<sub>Diet*Time</sub> = 0.479; p<sub>Treatment*Time</sub> < 0.001. *: Bars within a diagram lacking a common superscript differ (p < 0.05).
Figure 4. Daily nitrogen (N) retention (mg N/kg\(^{-0.67} \cdot d\)) during 5 days of physiological conditions ante injectionem (a. inj.) and 2 days post injectionem (p. inj.) in phylogenetic diverse pullets each selected for high [white WLA, a] and brown BLA, b) and low [white R11, c] and brown L68, d) daily egg mass production supplied with low (LA), adequate (AA) and high (HA) dietary L-arginine and treated with 2 mg \textit{E.coli} lipopolysaccharide (LPS) per kg BW or 0.9 % saline solution (NaCl; LSMeans ± SE; n = 3). ANOVA (\(p\) values): \(p\) Genotype < 0.001; \(p\) Diet = 0.524; \(p\) Treatment < 0.001; \(p\) Time < 0.001; \(p\) Genotype*Diet = 0.972; \(p\) Genotype*Treatment = 0.633; \(p\) Genotype*Time < 0.01; \(p\) Diet*Treatment = 0.450; \(p\) Diet*Time = 0.778; \(p\) Treatment*Time < 0.001. \(a\)-\(d\): Bars within a diagram lacking a common superscript differ (\(p < 0.05\)).
Core body temperature
The genotypes showed an average CBT of 41.5 ± 0.1 °C during light period (0500 to 1600 h) and 40.6 ± 0.1 °C during night period (1600 to 0500 h), and did not differ statistically from each other under physiological conditions (days 1 to 5). The CBT (Figure 5) was affected by “genotype”, “treatment” and “time” as well as their two- and three-factorial interactions ($p < 0.001$) during immune challenge. An effect of dietary Arg supply was not found ($p_{\text{Diet}} = 0.189$). The genotypes showed physiological CBT before treatment and after saline administration. However, LPS induced alterations of physiological CBT ($p_{\text{Treatment}} < 0.001$). Temperature profiles and amplitudes of fever differed between genotypes ($p_{\text{Genotype}} < 0.001$). Brown genotypes showed a severe hypothermia (L68: 40.2 °C; BLA: 40.7 °C) in the first 4 hours after LPS administration compared with white genotypes ($p_{\text{Genotype} \times \text{Time}} < 0.001$). Afterwards CBT increased and BLA developed fever ranging from 42.2 to 42.6 °C from 9 to 19 hours post inj. In the following BLA’s CBT approached the physiological course of saline treated pullets and did not differ from those pullets anymore. In contrast to BLA, L68 did not response as strong after initial hypothermia. The CBT of L68 elevated to 41.7 to 42.0 °C and approached to physiological CBT course after 24 hours, already. Whereas an initial hypothermia was absent in white genotypes, R11 showed a severe biphasic fever response at 4 h (up to 43.0 °C) and 11.5 h post inj. (up to 42.6 °C) which decreased strongly afterwards. Twenty-four hours after LPS injection the CBT returned to the physiological course as observed in the saline controls until the end of trial. In contrast to the other genotypes, WLA’s CBT did not respond to LPS during the first 8 hours post inj. At 11.5 hours post inj. CBT increased to 42.5 °C and slightly decreased to 41.7 °C afterwards, remained constant until 24 hours post inj. and re-increased up to 42.2 °C for 8 hours. In the following WLA’s CBT decreased strongly and approached its physiological course until the end of trial.

Clinical changes
During the entire trial no pullet died. The observed clinical changes (Table 2; Figure 6) describing pullets’ sickness behavior were affected by “genotype”, “treatment” and “time” as well as their two- and three-factorial interactions ($p < 0.001$). Arg supply did not influence sickness behavior ($p_{\text{Diet}} = 0.351$).

The pullets showed physiological behavior and general condition (score 0) before treatment. Clinical changes were absent in saline treated pullets during the entire trial (data not shown).
However, LPS produced alterations in pullet’s behavior ($p_{Treatment} < 0.001$). High performing WLA suffered most heavily from LPS, as their behavior and general condition showed the strongest deviation from the initial values and saline group ($p_{Genotype} < 0.001$). The other genotypes did not differ from each other in severity of body posture averagely ($p = 0.528$), whereas in BLA more intensive alterations in behavior and respiration were observed than in R11 and L68 ($p_{Genotype} < 0.001$), but BLA did not differ from L68 in plumage ($p = 0.247$).

Within 40 min $p. inj.$ of LPS pullets generally showed first changes in behavior ($p_{Treatment*Time} < 0.001$). The expression of sickness behavior peaked at 2 hours $p. inj.$ and persisted for 3 to 5 hours depending on treated genotype ($p_{Time} < 0.001$; $p_{Treatment*Time} < 0.001$). Afterwards symptoms subsided and reached initial values at 10.5 hours $p. inj.$ with the exception of body posture. Because the light period ended 9 hours $p. inj.$, pullets showed their physiological resting body posture, equivalent to score 1, until light period of the following day began. Afterwards pullets showed physiological body posture (score 0).

**Live body weight and relative weights of organs at 48 hours post-injection**

Table 4 summarizes the results of live BW and relative organ weights at slaughtering. The BW of pullets differed between the genotypes at the end of trial as it did before the experiment ($p_{Genotype} < 0.001$). Brown genotypes achieved a higher BW than white ones and LA caused a growth reduction in contrast to both Arg supplemented diets ($p_{Genotype} < 0.001$; $p_{Diet} < 0.01$). The relative weights of heart and gizzard (data not shown) were not affected by “genotype”, “diet” and “treatment”. However, in contrast to the other genotypes, WLA achieved higher relative weights of liver, pancreas, bursa and thymus, whereas L68 showed higher weights of spleen and cecal tonsils ($p_{Genotype} < 0.001$). Genotype R11 showed the lowest relative weights of spleen and thymus, but BLA had the lowest relative weights of pancreas and bursa ($p_{Genotype} < 0.001$). Relative weights of liver and pancreas were higher in LA fed pullets compared with AA and HA fed ones ($p_{Diet} < 0.05$). The LPS treatment induced higher relative weights of liver and spleen than saline ($p_{Treatment} < 0.001$), but relative weights of pancreas, bursa, thymus and cecal tonsils were lower 48 hours after LPS application in contrast to saline groups.
Figure 5. Time course of core body temperature from one hour ante injectionem to 48 hours post injectionem of 2 mg E. coli lipopolysaccharide (LPS) per kg BW or 0.9 % saline solution (NaCl) in phylogenetic diverse pullets each selected for high [white WLA, a) and brown BLA, b)] and low [white R11, c) and brown L68, d)] daily egg mass production and supplied with low (LA), adequate (AA) and high (HA) dietary L-arginine (LSMeans ± SE; n = 3).

ANOVA (p values): \( p_{\text{Genotype}} < 0.001; p_{\text{Diet}} = 0.243; p_{\text{Treatment}} < 0.001; p_{\text{Time}} < 0.001; p_{\text{Genotype}\times\text{Diet}} = 0.254; p_{\text{Genotype}\times\text{Treatment}} < 0.001; p_{\text{Genotype}\times\text{Time}} < 0.001; p_{\text{Diet}\times\text{Treatment}} = 0.268; p_{\text{Diet}\times\text{Time}} = 0.267; p_{\text{Treatment}\times\text{Time}} < 0.001.\)
Figure 6. Time course of sickness behavior from one hour *ante injectionem* to 13 hours *post injectionem* of 2 mg *E.coli* lipopolysaccharide (LPS) per kg BW or 0.9 % saline solution (NaCl) in phylogenetic diverse pullets each selected for high (white WLA and brown BLA) and low (white R11 and brown L68) daily egg mass production and supplied with low (LA), adequate (AA) and high (HA) dietary L-arginine (LSMeans ± SE; n = 3). ANOVA (p values): $p_{\text{Genotype}} < 0.001$; $p_{\text{Diet}} = 0.294$; $p_{\text{Treatment}} < 0.001$; $p_{\text{Time}} < 0.001$; $p_{\text{Genotype*Diet}} = 0.382$; $p_{\text{Genotype*Treatment}} < 0.001$; $p_{\text{Genotype*Time}} < 0.001$; $p_{\text{Diet*Treatment}} = 0.239$; $p_{\text{Diet*Time}} = 0.867$; $p_{\text{Treatment*Time}} < 0.001$. 
Figure 6 continued.
Plasma amino acids

The analyzed plasma amino acids were differently affected by “genotype”, “diet” and “treatment”. The sum of amino acids was lower in R11 plasma than in that of the other genotypes at 48 hours p. inj. (6271 ± 179 µM/L vs. 7368 ± 179 µM/L; \( p_{\text{Genotype}} < 0.001 \)) and LPS caused a lower sum of plasma amino acids than saline (6726 ± 127 µM/L vs. 7490 ± 127 µM/L, \( p_{\text{Treatment}} < 0.001 \)). As a number of concentration changes were found in analyzed plasma amino acids, we limited the graphical presentation (Figure 7) to those amino acids that were important for the interpretation of the trial and were affected by both “diet” and “treatment”, namely Arg, ornithine, citrulline and 3-methyl-histidine. Except for some genotype-specific differences in plasma amino acids (data not shown), changes in amino acid concentrations 48 hours p. inj. could be grouped as follows: **group I**: LPS caused plasma concentration decreases of alanine (-28 %), Arg (-23 %), asparagine (-35 %), citrulline (-16 %), cystathionine (-10 %), glutamic acid (-6 %), glycine (-10 %), hydroxyproline (-17 %), leucine (-11 %), methionine (-19 %), ornithine (-30 %), phenylalanine (-5 %), proline (-15 %), serine (-12 %), taurine (-27 %), tryptophan (-13 %) and tyrosine (-25 %; \( p_{\text{Treatment}} < 0.01 \)); **group II**: LPS caused plasma concentration increases of anserine (+12 %), aspartic acid (+15 %), β-alanine (+35 %), lysine (+18 %), 1-methyl-histidine (+21 %) and 3-methyl-histidine (+55 %; \( p_{\text{Treatment}} < 0.01 \)); **group III**: increasing concentrations of dietary Arg caused plasma concentration decreases of \( \alpha \)-amino adipic acid (-36 %), alanine (-24 %), anserine (-22 %), cystathionine (-10 %), glutamic acid (-9 %), glutamine (-25 %), glycine (-8 %), histidine (-16 %), phenylalanine (-10 %) and tyrosine (-27 %; \( p_{\text{Diet}} < 0.01 \)); **group IV**: increasing concentrations of dietary Arg caused plasma concentration increases of Arg (+128 %), citrulline (+58 %), ornithine (+650 %) and 3-methyl-histidine (+31 %; \( p_{\text{Diet}} < 0.001 \)). Amino acids showing only genotype-specific differences were carnosine, cysteine, isoleucine, threonine and valine.
Table 4. Live body weights and relative organ weights at 48 hours after intramuscular injection of 2 mg *E.coli* lipopolysaccharide (LPS) per kg BW or 0.9 % saline solution (NaCl) in phylogenetic diverse pullets each selected for high (white WLA and brown BLA) and low (white R11 and brown L68) daily egg mass production and supplied with low (LA), adequate (AA) and high (HA) dietary L-arginine (LSMeans, FSEM, n = 3).

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<th>Treatment</th>
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<th>Bursa</th>
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ANOVA (p values)
- **Genotype**
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001

- **Diet**
  - < 0.01
  - < 0.01
  - < 0.05
  - 0.168
  - 0.156
  - 0.347
  - 0.846
  - 0.168
  - 0.268
  - 0.268
  - 0.268
  - 0.268
  - 0.268
  - 0.268
  - 0.268
  - 0.268
  - 0.268
  - 0.268
  - 0.268

- **Treatment**
  - 0.104
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001
  - < 0.001

- **Genotype x Diet**
  - 0.416
  - 0.475
  - 0.436
  - 0.890
  - 0.676
  - 0.409
  - 0.841
  - 0.676

- **Genotype x Treatment**
  - 0.662
  - < 0.05
  - 0.101
  - 0.854
  - 0.074
  - 0.787
  - 0.800

- **Diet x Treatment**
  - 0.799
  - 0.957
  - < 0.05
  - 0.917
  - 0.654
  - 0.175
  - 0.146

*p* < 0.05: LSMMeans within a column lacking a common superscript differ (*p* < 0.05).

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Figure 7. Absolute plasma concentrations of four relevant amino acids at 48 hours post injection of 2 mg *E. coli* lipopolysaccharide (LPS) per kg BW or 0.9% saline solution (NaCl) in phylogenetic diverse pullets each selected for high (white WLA and brown BLA) and low (white R11 and brown L68) daily egg mass production and supplied with low (LA), adequate (AA) and high (HA) dietary L-arginine (LSMeans ± SE; n = 3). **”: Bars of a plasma amino acid lacking a common superscript differ (p < 0.05). ANOVA (p values): L-arginine - $p_{Genotype} = 0.067$; $p_{Diet} < 0.001$; $p_{Treatment} < 0.001$; $p_{Genotype*Diet} < 0.05$; $p_{Genotype*Treatment} = 0.956$; $p_{Diet*Treatment} = 0.118$; L-ornithine - $p_{Genotype} < 0.01$; $p_{Diet} < 0.001$; $p_{Treatment} < 0.01$; $p_{Genotype*Diet} < 0.05$; $p_{Genotype*Treatment} = 0.223$; $p_{Diet*Treatment} < 0.01$; L-citrulline - $p_{Genotype} < 0.01$; $p_{Diet} < 0.001$; $p_{Treatment} < 0.05$; $p_{Genotype*Diet} = 0.165$; $p_{Genotype*Treatment} = 0.280$; $p_{Diet*Treatment} = 0.572$; 3-methyl-histidine - $p_{Genotype} < 0.01$; $p_{Diet} = 0.068$; $p_{Treatment} < 0.001$; $p_{Genotype*Diet} = 0.196$; $p_{Genotype*Treatment} = 0.593$; $p_{Diet*Treatment} = 0.539$. 
Discussion

Pathogen associated molecular patterns, such as *E.coli* LPS, induces an acute-phase reaction accompanied by a dose-dependent moderate to severe systemic inflammation in birds (TAKAHASHI et al. 1995; XIE et al. 2000; DE BOEVER et al. 2008). The underlying mechanisms are regulated by inflammatory mediators and hormones, in domestic fowl factors similar to mammalian IL-1β and IL-6 (KLASING et al. 1987; KLASING and PENG 1990; KLASING and JOHNSTONE 1991). Both cytokines directly affect the hypothalamus and activate hypothalamic-pituitary-adrenal axis, the key pathway for prostaglandin-induced fever, sickness behavior and anorexia (MACARI et al. 1993; KLASING 1994; XIE et al. 2000). In addition, IL-6 stimulates hepatic synthesis and secretion of acute-phase proteins in mammals and birds mainly (GRUYS and LANDMAN 1997; GRUYS et al. 2005), whereas TNF-α induces protein breakdown and muscle wasting in mammalian acute inflammatory response (COONEY et al. 1997). Although TNF-like activities are observed in chickens, an avian orthologue has not been found in birds to date (KAISER et al. 2005; KAISER and STAEHELI 2014). On the basis of these mechanisms we use the chicken model described by LIEBOLDT et al. (2015 a) for the purpose of establishing an avian acute-phase model for immunonutritive research. The design aims to induce a severe acute-phase response by intramuscular injection of 2 mg LPS per kg BW. The focus of interest lays on the effects of a long-term graded dietary Arg supply on physiological, behavioral and metabolic traits in 18-wk-old pullets of purebred layer lines differing in phylogeny and performance once immune stimulated with *E.coli* LPS. Because changes in CBT and sickness behavior are closely correlated with LPS-induced acute-phase reaction in chicken (TAKAHASHI et al. 1995; XIE et al. 2000; DE BOEVER et al. 2008), we consider our experimental design as well-suited to induce a generalized inflammation and acute-phase reaction in the studied genotypes.

The LPS-induced systemic inflammatory response is regulated by a number of mediator molecules from which the cytokines released into circulation are of pivotal importance. In the present study the LPS caused severe sickness behavior accompanied with a strong fever response. These clinical alterations can be regarded as indicators for the activities of IL-1β, IL-6 and type I interferon in avian acute-phase reaction (MACARI et al. 1993; XIE et al. 2000; WIGLEY and KAISER 2003; DE BOEVER et al. 2008). The sickness behavior was characterized by anorexia, lethargy, ruffled feathers and intensified respiration for
approximately 12 hours that is comparable to the findings of KOH et al. (1996), XIE et al. (2000) and CHENG et al. (2004). These authors have described drowsiness, lethargy and anorexia that begin in the first hour after LPS injection and persist for 4 hours. Ruffled feathers and moderate diarrhea was observed temporarily and birds recovered completely by 24 hours p. inj.. In contrast to graded dietary Arg feeding, pullet’s genotype influenced the severity of sickness behavior. Although high performing genotypes expressed sickness behavior stronger than low performing genotypes, pullets generally show a similar behavioral response to LPS challenge. Sickness behavior is described as the expression of a complex interaction of the nervous, endocrine and immune system, in which especially chief pro-inflammatory cytokines IL-1β, IL-6 and TNF are involved in facilitating connections among all three systems and inducing sickness behavior centrally (BESEDOVSKY and DEL REY 1996, 2001; DANTZER 2001). CHENG et al. (2004) have suggested that basic mechanisms of behavioral responses to LPS are not altered through selection in genotypes and that sickness behavior has a common phylogenetic origin. Furthermore, the recognizable changes in bird’s behavior were accompanied by a measureable fever response. GREGORUT et al. (1992) and FRAIFELD et al. (1995, 1998) have reported on avian fever response after LPS administration and that mechanisms of fever in birds are similar to those in mammals. In order to create an inhospitable environment for invading pathogens, LPS induces strain and time dependent changes of CBT in pullets mediated by pro-inflammatory cytokines (LESHCHINSKY and KLASING 2001). Compared to saline, LPS induced an initial hypothermia in brown genotypes, followed by a severe hyperthermia in BLA and a slight hyperthermia in L68. On the contrary, white genotypes showed hyperthermia only. However, each genotype had a unique pattern of regulating CBT in response to LPS-induced immune stress and that could result from genotypes’ diverse capability to resist stress. White genotypes showed biphasic hyperthermia with longer-lasting hyperthermia in WLA and fast recovery in R11, whereas brown genotypes had a biphasic fever response with initial hypothermia and longer-lasting hyperthermia. Similar to the present results, previous studies demonstrate that LPS induces fever in chicks differently, such as monophasic hypothermia (SMITH et al. 1978) or a biphasic response divided in an initial phase of hypothermia followed by hyperthermia (ROTIROTI et al. 1981; DE BOEVER et al. 2008) or divided in a biphasic hyperthermia (JOHNSON et al. 1993; KOH et al. 1996;
XIE et al. (2000). Explanations for differences in avian fever response found between studies may result from differences in used pathogen, serotype, batch or dosage of LPS, its route of application as well as host’s sensitivity to LPS, its genetic background and age. Variations in sensitivity to LPS and its associated resistance or susceptibility to diseases are described for different chicken lines and strains. ABASHT et al. (2009) have reported on variations in Toll-like receptor expression among different lines. DIL and QURESHI (2002 b) have described further significant genetic line effects on splenic Toll-like receptor expression of Salmonella enteritidis infected chicken and the authors have assumed that underlying mechanisms are related to gene polymorphisms or varying numbers of receptors on immune cells.

Based on the successful induction of systemic inflammatory response, indicated by the dietary independent fever and clinical response in our genotypes, the immune modulating properties of dietary Arg were examined closer in the weights of organs, N balance and plasma amino acids. Insufficient dietary Arg caused lower BW as well as higher relative weights of liver and pancreas probably in response of increased hepatic and pancreatic enzyme activities. In accordance to our findings in 18-wk-old pullets, KIDD et al. (2001) and DENG et al. (2005) have not found an effect of dietary Arg on the weights of lymphoid organs in growing turkeys and Leghorn-type chicks. On the contrary, KWAK et al. (1999, 2001) have reported on lower weights of lymphoid organs in deficiently Arg fed 2-wk-old White Leghorns. These contradicting results lead to the assumption that poultry differs in its sensitivity to dietary Arg affecting organ development rather in a breed-specific than in an age-specific manner. However, in LPS-induced acute-phase reaction liver and spleen showed higher relative weights on the one hand, and pancreas and lymphoid organs, bursa and thymus, as well as cecal tonsils decrease in their relative weights on the other hand. It can be concluded, that liver and spleen may responded to LPS by increasing production and secretion of several proteins, mediators and cells. XIE et al. (2000) and CHENG et al. (2004) have described LPS-induced increases in liver’s metabolic functions such as gluconeogenesis, glucose oxidation, synthesis of fatty acids and the elevated release of acute-phase proteins and cytokines including IL-1β. The increase of relative spleen weight emphasizes its importance for immune response (CHENG et al. 2004; SHINI et al. 2008), because elevated weights of immune organs are associated with enhanced immune cell replication and proliferation. Although indications for blood congestion were not macroscopically visible in the examined livers and
spleens, from porcine acute-phase models it is known that LPS induces hepatic leucocyte infiltration, oedema and sinusoidal dilatation (SAETRE et al. 2001; MARTENS et al. 2007). In contrast to SHINI et al. (2008), who have not found any alterations in relative bursa weights, the present study showed a decrease in relative weight of the bursa Fabricii in LPS treated pullets. RIDDELL (1987) has assumed that the bursa Fabricii is sensitive for a stress-induced bursal atrophy caused by increased corticosteroid production during endotoxemia. Additionally, decreased weights of the bursa Fabricii, thymus and cecal tonsils suggest a release of immune cells from these lymphoid organs into circulation probably. Moreover, the daily N balance as well as amino acid analysis of a single plasma sample are considered to be sensitive characteristics for the evaluation of whole body N metabolism in healthy and endotoxemic pullets, because N balance is classified to be very important in nutrition and metabolic management of critical ill humans (KONSTANTINIDES 1992). The dietary Arg supplementation influences avian immune function (KWAK et al. 2001; DENG et al. 2005; TAN et al. 2014) whereas Arg has the highest N proportion from all proteinogenic amino acids. The present study revealed no effect of dietary Arg level on DWG, DFI, N intake and N retention in saline and LPS treated pullets. To the best of our knowledge comparative studies in avian species are not existent. However, BRUINS et al. (2002) have not found N balance improving effect of parenteral Arg supplementation in endotoxemic pigs, whereas SAITO et al. (1987), BRAGA et al. (1996, 1999), and CUI et al. (1999) have reported on limitation of protein and BW loss during injury or surgery in rats as well as humans by Arg supplementation. In our study, pullets’ N metabolism responded in two steps to the administrated LPS: during the first 24 hours LPS induced a strong catabolic phase characterized by negative N balance accompanied with BW loss and anorexia. In the second 24 hours LPS treated pullets metabolically recovered and became anabolic as shown by positive N retention, DWG and increased feed and N intake. However, pullets did not regained baseline values and concentrations of certain required amino acids were decreased in plasma irrespective of genotype. Due to the metabolic activation of immune system and the sickness-induced reduction of feed intake, a generalized dysregulation of nutrient homeostasis occurs and enhances imbalances in required amino acids in endotoxemia (ELSASSER et al. 2000; HUMPHREY et al. 2002), which are underlined by a decreased plasma concentration of total amino acids (BRUINS et
al. 2002). The authors have showed further decreases of Arg, glycine, glutamine, tyrosine and the sum of the branched chain amino acids in endotoxemic pigs, whereas LUIKING and DEUTZ (2007) have described decreases of Arg, citrulline, glutamate, glutamine, lysine, ornithine, threonine and valine in endotoxemic humans. As a consequence, these amino acids may be released from skeletal muscle into circulation in order to provide required amino acids for hepatic gluconeogenesis and synthesis of acute-phase proteins as well as for further immunological processes such as immune cell replication and proliferation (KLASING and AUStIC 1984; KLASING 1988; BARNES et al. 2002). In mammals, COONEY et al. (1997) have reported on the LPS-induced release of TNF-α, which induced protein breakdown and muscle wasting during acute-phase reaction consecutively. Although a possible chicken TNF-α orthologue has not been identified to date (KAISER et al. 2005; KAISER and STAHELI 2014), the LPS-induced negative N balance and increased concentrations of plasma 3-methylhistidine, anserine and β-alanine indicate an inflammatory protein breakdown and muscle wasting that might be mediated through TNF-like activities in chickens, too. Plasma 3-methylhistidine is described as sensitive indicator for proteolysis of muscle protein associated with decreased N retention in acute-phase reaction (ZAMIR et al. 1992; LOI et al. 2005). Because β-alanine, a component of endogenous antioxidants anserine and carnosine, is highly concentrated in muscle tissues (SALE et al. 2010), a release of β-alanine and anserine from muscles elevates their plasma concentrations and may indicate LPS-induced muscle wasting and protein breakdown further. In accordance to our findings, the acute-phase reaction also decreases the plasma concentrations of aromatic amino acids such as phenylalanine, tryptophan and tyrosine, as they are needed for hepatic synthesis of acute-phase proteins (REEDS et al. 1994). As LPS-treated pullets suffered from anorexia, energy and amino acids are not provided from ingested feed into circulation anymore. Consequently, pullet’s metabolism might change to hepatic gluconeogenesis in order to support organs and tissues with required energy (ROSENBLATT et al. 1983; SAX et al. 1988). Hepatic gluconeogenesis partly utilizes Krebs citrate cycle intermediates such as pyruvate, oxalacetate, acetyl-CoA and α-ketoglutarate, which can be generated from several glucoplastic amino acids such as alanine, Arg, asparagine, glutamic acid, glycine, methionine and proline. In the present study the LPS-induced decline in plasma concentrations of these amino acids might indicate the increased hepatic synthesis of acute-phase proteins and
gluconeogenesis in pullets. Consequently, limited dietary amino acid influx and increased consumption during acute-phase reaction would lower their total plasma concentration. The LPS-induced decrease in plasma Arg concentration could result further from an Arg consumption for immunomodulatory functions, which comprise the improvement of directly cytotoxic mechanisms via Arg-NO pathway as well as enhancement of immune cell proliferation, cell division and DNA replication via Arg-ornithine-polyamine pathway (LUKING et al. 2005; KHAJALI and WIDEMAN 2010).

In addition to LPS-induced changes, graded dietary Arg supply levels caused significant alterations in plasma amino acid concentrations. The decrease of certain amino acids in the plasma of pullets fed with increasing Arg concentrations can be understood as compensatory mechanism. In accordance to CHU and NESHEIM (1979), KWAK et al. (1999) and LOI et al. (2005) increasing amounts of dietary Arg elevate plasma Arg directly causing secondary increase of plasma ornithine concentrations and, in our study, even elevated plasma citrulline and 3-methyl-histidine concentrations. Because arginase and iNOS compete for Arg as a common substrate in acute phase reaction, it can be suggested that higher amounts of available plasma Arg may be beneficial for host’s immune response as described by LEE et al. (2002), DENG et al. (2005) and TAN et al. (2014).

In conclusion, we have demonstrated that bacterial LPS acts as a potent stimulator of avian immune system and induces strong alterations in chicken’s sickness behavior especially anorexia and CBT as well as severe changes in N metabolism, plasma amino acids, and relative weights of digestive and immune organs. However, long-term graded Arg supply ranging from insufficient to oversupplied concentrations of recommendation (NRC 1994) only affects concentrations of certain plasma amino acids, BW and relative weights of digestive organs. As Arg does not affect significantly further physiological and behavioral traits in the present study, it can be concluded that the Arg requirement for these examined traits is equal to or less than the lowest level used in this study, which has been 70 % of recommended Arg (NRC 1994). In particular, the results of the N balance trial do not indicate that our insufficient dietary Arg level was limiting for N retention. On the other hand, the studied traits may be not specific or sensitive enough to detect Arg effects on immunological and metabolic responses as found by KWAK et al. (1999), DENG et al. (2005) and TAN et al.
Consequently, further research shall focus on cellular immunology in the established animal model and experimental design.

Acknowledgments
The authors gratefully acknowledge Mrs. A. Junghans (Institute of Animal Nutrition, Friedrich-Loeffler-Institute, Braunschweig, Germany) for the care of the experimental animals, the sample and data collection and sample preparation as well as the practical realization of the experiment. The authors acknowledge further Mr. H. Eckardt (Institute of Animal Nutrition, Friedrich-Loeffler-Institute, Braunschweig, Germany) for the analysis of feed and excrement samples.

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The maximum implementation of a genetically determined performance potential requires the optimal allocation and utilisation of available metabolic resources in livestock species in order to cope adequately with the surrounding abiotic and biotic environment (HAVENSTEIN et al. 2003; MATHUR 2003; CHENG and MUIR 2005). However, the selection for high production efficiency is suspected to cause a shift of endogenous resources away from fitness-related traits towards performance-related traits with a subsequent unbalanced use of these resources (BEILHARZ et al. 1993; RAUW et al. 1998). The selection causes high performing genotypes with a reduced resource capacity to compensate unexpected environmental changes such as nutritional limitations (VAN DER WAAIJ 2004; MIRKENA et al. 2010). For that reason, a maintained high performance would lead to metabolic stress merging into health disorders under varying or even suboptimal nutritional conditions (MIRKENA et al. 2010).

Among the large variety of abiotic environmental factors, dietary Arg takes an essential role in growth and performance (CUCA and JENSEN 1990; KWAK et al. 1999; WU et al. 2010) as well as immune response of chickens (KWAK et al. 1999, 2001; TAYADE et al. 2006 a, b; TAN et al. 2014). Therefore, the present thesis investigated the genotype-dependent adaptability of layer-type chickens to variations of dietary Arg on the basis of their growth and performance response from hatch onwards (Paper II and III). The experimental fundament was formed by diets containing Arg equivalent to 70, 100 and 200 % of recommended supply (NRC 1994) and by a chicken model (Figure 6) consisting of four purebred layer lines differing in their phylogenetic origin (white vs. brown) and performance level (high vs. low; Paper I). On the assumption that a further exposure to a biotic environmental stress factor could intensify the adaptive difficulties of chickens to dietary Arg, the metabolic and immunological response to a single E.coli LPS injection of 2 mg LPS per kg BW i.m. was examined in reared chickens additionally (Paper IV and V).

First of all, the critical view on the conducted research verified the established chicken model and the gradation of dietary Arg as suitable experimental tools for testing the postulated hypotheses. Since GRANEVITZE et al. (2007, 2009) and LYIMO et al. (2014) have reported on the phylogenetic distant relationship between white layers and brown layers, Paper I and LIEBOLDT et al. (2015) confirmed the assumed, marked differences between selected layer
lines (WLA and BLA) and non-selected layer lines (R11 and L68) in their genetically determined performance potential under commercial feeding conditions. However, the suitability of this model has to be differentiated between the rearing period and the laying period (Paper I). While the rearing period was predominated by the phylogenetic divergence in growth related traits, the aspired performance divergence came to the fore in the relevant performance related traits during the laying period only (Paper I).

Due to chicken’s inability of synthesising Arg de novo (TAMIR and RATNER 1963 a), available plasma Arg directly depends on the provided dietary Arg supply in chickens (CHU and NESHEIM 1979; KWAK et al. 1999, 2001). This relationship was confirmed by the analysis of diets (Paper II to V) and plasma amino acids in pullets (Paper V) considering the effective realisation of the intended dietary Arg gradation in reared chickens. However, the dietary effects observed in laying hens (Paper II) had to be reviewed more critically than those in reared chickens. Whereas the provided dietary crude protein was sufficient to implement the genetically determined performance potential in low performing genotypes, these diets failed to supply the high performing genotypes with crude protein adequately and led to severe metabolic disorders.

Both studies described in Paper II and III were used to test the postulated hypothesis that a marginal dietary Arg supply induces adaptive difficulties in growth and laying performance in high performing layer-type chickens more pronounced than in low performing ones. In general, these studies confirmed the indispensable character of dietary Arg for the growth of chickens during the rearing period. In accordance to KWAK et al. (1999), LEE et al. (2002), DENG et al. (2005), JAHANIAN (2009) and WANG et al. (2014) insufficient dietary Arg caused depressions in feed intake and growth secondary. In contrast to KIDD et al. (2001) and WANG et al. (2013) reporting on growth enhancing effects of dietary Arg beyond recommendations of NRC (1994) in meat-type poultry, the present studies gave no indication for further growth promotion by surplus dietary Arg (Paper II and III). Due to chickens’ inability of synthesising Arg de novo (TAMIR and RATNER 1963 a), the adverse effects of insufficient dietary Arg derived from the reduced availability of plasma Arg (CHU and NESHEIM 1979; KWAK et al. 1999, 2001; Paper V) and might possibly include dysregulation of pathways involved in regulation of feed intake and growth as described in detail in the background of the thesis.
Table 3. Genotype-specific expression patterns of growth, performance and egg quality related traits as well as relative organ weights induced by graded L-arginine supply (Reference: Paper II and III, grey, red, yellow and green coloured boxes: No differences between diets and lowest, intermediate and highest expression of trait within a genotype.

<table>
<thead>
<tr>
<th>Genotype x diet</th>
<th>WLA</th>
<th>BLA</th>
<th>R11</th>
<th>L68</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LA</td>
<td>AA</td>
<td>HA</td>
<td>LA</td>
</tr>
<tr>
<td>Growth related traits during rearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW</td>
<td>red</td>
<td>green</td>
<td>red</td>
<td>green</td>
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<tr>
<td>DWG</td>
<td>red</td>
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<tr>
<td>EABW</td>
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<td>t\textsubscript{max}</td>
<td>green</td>
<td>red</td>
<td>green</td>
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</tr>
<tr>
<td>MDWG</td>
<td>red</td>
<td>green</td>
<td>red</td>
<td>green</td>
</tr>
<tr>
<td>DFI</td>
<td>red</td>
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<td>green</td>
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<tr>
<td>FCR</td>
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<tr>
<td>Relative organ weights during rearing</td>
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<tr>
<td>Bursa</td>
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<td>Gizzard</td>
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<td>Heart</td>
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<td>Liver</td>
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<tr>
<td>Pancreas</td>
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<td>Spleen</td>
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<td>Thymus</td>
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<tr>
<td>Performance related traits during laying period</td>
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<td></td>
</tr>
<tr>
<td>BW</td>
<td>red</td>
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<tr>
<td>DFI</td>
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<td>LI</td>
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<td>FEM</td>
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<tr>
<td>Egg quality related traits</td>
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<tr>
<td>Albumen p.</td>
<td>red</td>
<td>green</td>
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<tr>
<td>Shell p.</td>
<td>red</td>
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<tr>
<td>Yolk p.</td>
<td>red</td>
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<tr>
<td>Yolk colour</td>
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</table>

LA, AA, HA: low, adequate and high Arg diet (equivalent to 70, 100 and 200 % of recommended Arg level (NRC, 1994)); BW: body weight; EABW: estimated adult BW; DWG: daily weight gain; MDWG: maximum DWG; t\textsubscript{max}: age at MDWG; DFI: daily feed intake; FCR: feed conversion ratio; LI: laying intensity; EW: egg weight; DEM: daily egg mass; FEM: feed to egg mass ratio; p.: proportion

In order gain a complete overview of the genotype-specific adaptability to the graded dietary Arg supply (Paper II and III), the expression patterns of traits related to growth, relative organ weights, performance and egg quality are summarized in Table 3 for each genotype. Independent of their genetic background, reared chickens grew with adequate and surplus dietary Arg equally, and responded to insufficient dietary Arg with growth depression and growth retardation in a similar manner. Within the studied genotypes, reared WLA and L68 tended to have greater difficulties to adapt to deficient dietary Arg because their feed intake
and maximum daily weight gain were adversely affected additionally (Paper II). Because both genotypes originated from different phylogenetic origins and performance levels (Figure 6), a directional interaction between the genetically determined performance potential and the graded dietary Arg supply was missing during the rearing period (Paper II and III). That leads to the assumption that the sensitivity of growth to deficient dietary Arg is genetically not linked to chickens’ performance potential and phylogenetic origin. The variations in growth observed between genotypes could indicate possible genotype-specific differences in Arg requiring processes such as feathering (HEGSTEDT et al. 1941; BEQUETTE 2003), appetite regulating NO production (KHAN et al. 2007; WANG et al. 2014) and the synthesis of creatine, polyamines and L-proline on the one hand (AUSTIC and NESHEIM 1971, 1972; GRILLO et al. 1978; CHAMRUSPOLLERT et al. 2002) and differences in Arg degrading and antagonising processes like the lysine metabolism and the activity of renal arginase on the other hand (HUTT and NESHEIM 1966; AUSTIC and NESHEIM 1970). As a consequence of these research findings, the first postulated hypothesis has to be rejected for the growth response of layer-type chickens.

Furthermore, the laying period described in Paper II aimed to test the performance aspect of the first postulated hypothesis. But as a result of the severe protein malnutrition in high performing genotypes, recorded data of laying performance cannot be reliably used to affirm or reject the validity of this hypothesis conclusively. However, the great adaptive difficulties of high performing genotypes to the limitations in dietary crude protein were impressively illustrated by a poor feed intake, strong mobilisation of body mass and low laying performance similar to that of low performing genotypes (Paper II). On the contrary, latter ones achieved a laying performance and BW development comparable with that described under commercial feeding conditions (Paper I). As R11 and L68 were sufficiently supplied with dietary crude protein, their laying performance indicated performance depressing effects of deficient dietary Arg similar to the growth depressing effects of marginal dietary Arg during the rearing period.

Nevertheless, this experiment gives some interesting information about the responsiveness of genetically diverse laying hens to low dietary crude protein when the results are put into the context of the resource allocation theory (BEILHARZ et al. 1993). Due to the unbalanced use of resources between fitness-related and performance-related traits (BEILHARZ et al. 1993),
RAUW et al. (1998) and MIRKENA et al. (2010) have hypothesised that high performing genotypes possess a reduced resource capacity to respond to environmental stress adequately. During an insufficient resource intake, the genetically determined priority of performance intensifies the unbalanced resource allocation leading to metabolic stress and health disorders subsequently (BEILHARZ et al. 1993; MIKRENA et al. 2010). In case of a deficient dietary crude protein supply, the strong mobilisation of body mass and the associated maintenance of laying performance support the idea for the genetically determined priority of performance in WLA and BLA compared with fitness (Paper II). In contrast to fitness prioritising adaptive mechanisms such as reduction of metabolism and termination of egg laying (MIRKENA et al. 2010), the acceptance of metabolic stress and subsequent health disorders in high performing layer lines has to be considered as undesirable side-effect of selection as mentioned by RAUW et al. (1998).

Moreover, the second part of the thesis tested the hypothesis that the metabolic and immunological response to an APR is more pronounced by a dietary Arg supply beyond the requirement for optimal growth and performance in high performing layer lines when compared to low performing strains. In the 12th and 18th week of age the genotypes of the established chicken model were challenged with *E. coli* LPS (Paper IV and V). Based on the facts that reared chickens are exposed to numerous environmental stress factors (DAVISION et al. 1983; SHINI et al. 2009) and that pullets varying in their later production efficiency differ markedly in their immunological response (VAN EERDEN et al. 2004), the present LPS challenges were performed in the late rearing period. According to literature findings (TAKAHASHI et al. 1995; XIE et al. 2000; CHENG et al. 2004; SHINI et al. 2008; TAN et al. 2014), a single parenteral injection of *E. coli* LPS was sufficient to trigger a systemic APR accompanied by severe alterations in behaviour, body temperature, differential blood cell counts, and various metabolic changes in nitrogen balance, concentration of plasma amino acids and relative weights of internal organs (Paper IV and V). After the pathophysiological mechanisms underlying the interaction of chickens’ genetic background and LPS were already extensively described and discussed in the corresponding sections of Paper IV and V, the following section focuses on the metabolism and immune response modulating effects of graded dietary Arg in the chicken genotypes observed in the course of the LPS challenges.
First of all, the present LPS challenges induced marked alterations in chickens’ body temperature and behaviour, which are closely correlated with the LPS-induced APR in chickens (Takahashi et al. 1995; Xie et al. 2000; Leshchinsky and Klasing 2001; De Boever et al. 2008). Sickness behaviour and fever response are described as the expression of a complex interaction of the nervous, endocrine and immune system, in which the chief pro-inflammatory cytokines facilitate the connections among all three systems and induce both clinical signs centrally (Be sedovsky and de rey 1996, 2001). According to relevant literature on avian and mammalian species, dietary Arg did not affect the sickness behaviour and fever response directly (Paper IV and V). As the response and expression of both clinical signs depend on the magnitude of centrally acting pro-inflammatory cytokines (Be sedovsky and de rey 1996, 2001; Stae heli et al. 2001), dietary Arg could modulate sickness behaviour and fever response indirectly by altering the release of pro-inflammatory cytokines per se. Serving as pivotal precursor of growth-promoting polyamines (Pegg and McCann 1982; Smith 1990) and possessing secretagogue activities that stimulate the release of immune cells affecting pituitary hormones (Barbul 1986; Dorskind and Horseman 2000; Calder and Yaq oob 2004), Arg could influence the proliferation of leukocytes and their subsequent release of pro-inflammatory cytokines indirectly in course of an immune stimulation. Those Arg-induced changes in the function of leukocyte may alter the leukogram.

In parallel with the LPS-induced sickness behaviour and fever response of chickens, severe changes in WBC counts were observed in accordance to the results of Latimer et al. (1988), Harmon (1998), Xie et al. (2000) and Wang et al. (2003). In the present study dietary Arg influenced neither the course of this haemoresponse nor the relative WBC proportions. On the contrary, studies on Arg oversupplied broiler chicks describe Arg-dependent elevations in the relative proportions of heterophils, T lymphocytes and monocytes after viral vaccination (Lee et al. 2002; Abdukalykova et al. 2008; Jahanian 2009; D’amato and Humphrey 2010). However, the comparability of haematological studies in physiological and pathophysiological birds is quite difficult because the physiological haemoresponse depends on a large variety of influencing factors including environment and management practices (Fourie and Hattin gh 1980; Wang et al. 2003; Clark et al. 2009). Nevertheless, the differences found between the present study and the mentioned
studies on broilers could result from the variations in the type of used pathogen as well as the duration and level of Arg supply on the one hand and a diverse amino acid requirement of fast growing broiler chicks leading to an age-dependent and genotype-specific higher susceptibility to the immunomodulating properties of dietary Arg on the other hand. In contrast to the effects of surplus dietary Arg in broilers, the total leukocyte counts increased in deficiently Arg supplied chickens independent of their genetic background (Paper IV). Although a mere increase of total leukocyte counts is a very unspecific parameter of the immune response, the present study pointed out deficient dietary Arg as further immunological stress factor in the course of a LPS-induced acute phase reaction.

In modern poultry medicine the H/L ratio serves as reliable and accurate physiological indicator for avian stress response (GROSS and SIEGEL 1983). Independent of the genetic background, the proportions of heterophils and lymphocytes behaved reciprocal under stressful conditions as described by DAVISON et al. (1983) and GROSS and SIEGEL (1983) and indicated a high-degreed stress response to LPS with reference to the authors’ stress classification. According to LEE et al. (2002) reporting on increased H/L ratios in Arg oversupplied broilers, the present study revealed that both insufficient and surplus dietary Arg tended to intensify the H/L ratios of chickens (Paper IV). That leads to the assumption that dietary Arg below and beyond NRC (1994) recommendations might serve as nutritional stress factor influencing the immune response. In addition to possible indirect effects of dietary Arg on the immune cells through its secretagogue activities mentioned above (BARBUL 1986; DORSHKIND and HORSEMAN 2000; CALDER and YAQOOB 2004), chicken mononuclear immune cells respond to variations in the availability of Arg by alterations in the expression of Arg importing and exporting membrane transporters (D’AMATO and HUMPHREY 2010). Therefore, an enhanced cellular Arg import or even export might induce metabolic stress in immune cells and cause the observed alterations in total leukocyte counts and H/L ratio subsequently.

Beside the immune cells, internal organs of chickens also express Arg transporters on their surface in an organ and age-specific manner (HUMPHREY et al. 2004; HUMPHREY and KLASING 2005). These organ-specific expression patterns of cationic amino acid transporters could further differ between chicken genotypes explaining the different sensitivities of organs and genotypes to dietary Arg. In the present study, the liver, pancreas
and bursa of Fabricius responded most sensitive to deficient dietary Arg with a marked increase in their relative weights during the rearing period, whereas the relative spleen weight decreased in chickens fed with surplus dietary Arg (Paper III). Referring to the allometric coefficient of organs, the strong growth retardation in lymphoid organs emphasised their high sensitivity to deficient dietary Arg additionally (Paper III). In comparison with other studies, partially contradictory statements on the sensitivity of chickens’ lymphoid organs to dietary Arg can be found. Whereas KIDD et al. (2001) and CHENG et al. (2004) have not observed any effect of graded dietary Arg on the growth of lymphoid organs in broiler chickens, KWAK et al. (1999) and JAHANIAN (2009) have reported on decreased relative weights of thymus and spleen in deficiently Arg supplied layer-type chickens and on increased relative weights of thymus and spleen in Arg oversupplied broiler chickens, respectively. Despite the differently directed response of lymphoid organs, the present results and those described in literature emphasise the high sensitivity of lymphoid organs to nutritional and immunological stress. For that reason, the size and weight of lymphoid organs is frequently used as very sensitive indicator of stress (RIDDELL 1987; SHELAT et al. 1997; PUVADOLPIROD and THAXTON 2000).

In addition to the immunological and behavioural changes, the sickness-induced anorexia and the metabolic activation of the immune system further dysregulate metabolic homeostasis during early inflammation (ELSASSER et al. 2000; HUMPHREY et al. 2002). Within 24 hours chickens responded to LPS with severe anorexia accompanied by a strong loss of body weight and negative nitrogen balance (Paper IV and V). Both alterations reflect the endogenous mobilisation of energy and amino acids through protein breakdown in skeletal muscles and lipid degradation (ROSENBLLATT et al. 1983; BARACOS et al. 1987; SAX et al. 1988; COONEY et al. 1997). This process aims at providing adequate amounts of nutrients required for the replication and proliferation of immune cells, the synthesis of acute phase proteins (GRUYS and LANDMAN 1997; GRUYS et al. 2005) and the realisation of a sufficient fever response (BARACOS et al. 1987; CHIOLÉRO et al. 1997). As the pattern of amino acids required in APR differs from that released by the protein breakdown (KLASING and AUSTIC 1984; KLASING 1988; BARNES et al. 2002), an increased nitrogen excretion with subsequent decreased nitrogen retention follows (DICKERSON et al. 1997, 2001; BREUILLLE et al. 1999; BRUINS et al. 2002). But interestingly, studies on mammals...
suffering from APR have reported on net nitrogen retention and improvements of hepatic and muscular protein metabolism when additional dietary Arg has been orally supplied (BARBUL 1990; EFRON and BARBUL 1998; EVOY et al. 1998; BRUINS et al. 2000, 2002; LUIKING et al. 2005). These muscle protein sparing and nitrogen balance enhancing effects of dietary Arg derived from its high nitrogen proportion (BRAGA et al. 1999; CUI et al. 1999). However, studies on those interacting effects of dietary Arg and LPS on chickens N balance do not exist to the best of the author’s knowledge and the present study found neither beneficial nor adverse effects of a graded dietary Arg supply on the N balance of pullets suffering from LPS-induced APR (Paper V).

In contrast to the 18-week-old pullets, the LPS-induced loss of body weight was intensified by deficient dietary Arg in 12-week-old cockerels independent of their genetic background (Paper IV). This diverse response to dietary Arg in chickens suffering from LPS could be explained by age-dependent variations in Arg requiring processes such as growth, feathering and feed intake. As cockerels showed a higher daily weight gain in their 12th week of age than 18-week-old pullets, it can be assumed that younger birds have a higher dietary Arg requirement leading to a higher susceptibility to dietary limitations of Arg expressed in the smaller pool of available plasma Arg of deficiently Arg supplied chickens (CHU and NESHEIM 1979; KWAK et al. 1999; Paper V). Based on the LPS-induced decrease of available plasma Arg observed in pullets (Paper V), it can be concluded that the sickness-induced anorexia (NIRGIOTIS et al. 1991; BRUINS et al. 2002; SUCHNER et al. 2002; LUIKING et al. 2005) as well as the Arg requiring immunological processes of the Arg-NO pathway (SUNG et al. 1991; KWAK et al. 2001) and of the Arg-Orn-polyamine pathway (LUIKING et al. 2005; KHAJALI and WIDEMAN 2010) exacerbate the limited pool of available plasma Arg in chickens further. Additionally, available plasma Cit and Orn elevated parallel to increasing amounts of dietary Arg indicating enhanced Arg degradation through the competing mechanisms of arginase and NOS activity in chickens (KHAJALI and WIDEMAN 2010). Although the present experiments did not reveal beneficial effects of surplus dietary Arg in metabolic and immunological response to LPS per se, it can be suggested that a higher availability of plasma Arg as provided by surplus dietary Arg may have advantageous impacts on chicken’s immune response as described by LEE et al. (2002), DENG et al. (2005) and TAN et al. (2014).
To sum up the findings of Paper IV and V, chickens’ metabolic and immunological response to LPS was not more pronounced in the high performing genotypes than in the low performing genotypes by a dietary Arg supply beyond the requirement for optimal growth and performance. On the contrary, the present study even emphasised the adverse effects of deficient dietary Arg on several metabolic and immunological processes by the intensification of LPS-induced alterations independent of chickens’ genetic background. Therefore, the second hypothesis has to be rejected, too.

In conclusion, the research conducted for this thesis has provided an important insight into the metabolic and immunological response to a long-term graded dietary Arg supply and an additional single exposure to *E.coli* LPS in layer-type chickens differing in phylogeny and performance. Depending on the missing directional interaction between the performance level of chickens and the graded dietary Arg supply in growth and performance response on the one hand and in the metabolic and immunological response on the other hand, the postulated hypotheses of the present thesis have to be rejected. However, the vast majority of examined parameters were affected rather by single genotypes or those of the same phylogenetic origin than by those of the same performance level. Independent of chicken’s genetic background, the present research emphasised the essentiality of dietary Arg for chickens in their growth and performance response, characterised the divergent responsiveness and sensibility of several internal organs to dietary Arg and LPS, and identified deficient dietary Arg as a potential abiotic stress factor on avian metabolic and immunological response during LPS-induced APR.

Because the actual factors determining genotype-specific variations in the metabolic and immunological response to dietary Arg and LPS are still largely unknown, further research is needed to characterise them in detail. In order to get a deeper insight into the underlying mechanisms of organ and immune cell specific sensitivities to dietary Arg in chickens, studies should be carried out with more and smaller gradations of dietary Arg. In addition to the immune modulating effects of Arg on chicken’s innate immune response, these basic experiments should include examinations on the adaptive immune system of chickens such as investigations on divergent sensitivities of lymphocytes and their associated lymphoid organs to dietary Arg.
SUMMARY

Marc-Alexander Lieboldt (2015)

Effects of dietary L-arginine on metabolism and immune response in layer-type chickens of different genetic backgrounds under physiological and pathophysiological conditions

Since the early 20th century poultry industry has changed significantly in the fields of breeding, husbandry, nutrition, and health management. In this context the directional selection for a genetically determined high performance potential has increased the production efficiency in layer-type chickens markedly. However, this process is often associated with undesirable side-effects causing adaptive difficulties of chickens to their surrounding abiotic and biotic environment. For that reason, a suboptimal nutritional supply may cause metabolic stress merging into health disorders more pronounced in chickens selected for high production efficiency than in non-selected chickens. Among essential nutrients required by chickens, the amino acid L-arginine (Arg) plays a pivotal role in growth, performance, neurotransmission, vasoregulation and immune response. Thereby, a dietary Arg supply beyond the requirement for optimal growth elevates the availability of plasma Arg inducing immunomodulation in pathogen challenged chickens and improving nitrogen balance and protein metabolism of endotoxemic pigs, rodents and humans.

As the adaptability to varying dietary Arg has not been compared between genetically diverse layer-type chickens yet, the present thesis aimed to investigate the effects of a graded dietary Arg supply on growth, performance and immune response under physiological and pathophysiological conditions in chicken strains differing in phylogeny and laying performance.

The experiments of the present thesis based on a chicken model consisting of two white and two brown purebred layer lines with high and low laying performance each. In order to consider the age-dependent variations of nutritional requirements in layer-type chickens properly, chicks (hatch to week 7), pullets (week 8 to 16) and laying hens (week 16 to 41) were fed ad libitum with diets containing Arg equivalent to 70, 100 and 200 % of age-specific
SUMMARY

recommended supply. To evaluate the adaptability of the layer lines to dietary Arg in their growth and performance response under physiological conditions, 150 one-day-old female chicks of each line were distributed to the three diets for chicks equally. At the end of the rearing period 36 pullets of each experimental group were further used for the evaluation of performance during the laying period. In parallel with the rearing period, additional 12 one-day-old female chicks of each experimental group were reared under the same conditions separately to investigate genotype-specific organ growth as well as organ-specific sensitivities to dietary Arg in 6-weeks-intervals until the 18th week of age. During the rearing period insufficient dietary Arg caused a reduction of feed intake in high performing white and low performing brown chickens as well as a general growth depression and retardation in all four strains. However, the study gave no indication for further growth promotion by surplus dietary Arg in chickens. Insufficient dietary Arg increased the relative weight of the bursa, gizzard and liver and retarded the maturation of spleen and bursa. Yet, surplus dietary Arg decreased relative spleen weight. While the weights of heart, gizzard and liver decreased relatively to the body weight from hatch onwards, proportions of pancreas and lymphoid organs increased until week 12. The overall allometric evaluation of organ growth indicated a precocious development of heart, liver, gizzard, pancreas and bursa independent of chicken’s genetic and nutritional background.

In contrast to low performing genotypes, the high performing strains were unintentionally undersupplied with dietary crude protein regardless the grade of dietary Arg during the laying period. As a consequence, high performing hens showed a strong reduction of feed intake and laying performance followed by a severe mobilisation of body mass. On the contrary, low performing genotypes achieved their genetically performance potential and responded to deficient dietary Arg with a decline in laying performance supporting the conclusion that the protein requirement was met.

To evaluate the effects of a graded dietary Arg supply on the immune response of the four chicken genotypes under pathophysiological conditions, four 12-week-old cockerels and three 18-week-old pullets of each experimental group were challenged with an intramuscular injection of 2 mg E.coli lipopolysaccharide (LPS) per kg body weight in two separate experiments. In the study on pullets a saline treated, equally sized control group was examined additionally. In cockerels the effects of graded dietary Arg on LPS-induced
alterations of feed intake, weight gain, body temperature and differential blood counts were investigated over 48 hours post injection. In the following trial on pullets, the effects of graded dietary Arg on LPS-induced variations in weight gain, nitrogen balance, body temperature and behaviour were examined over 48 hours after LPS injection. In pullets the concentrations of plasma amino acids and the relative organ weights were one-time analysed at 48 hours post injection, too.

The differential blood counts in cockerels showed a severe leukopenia characterised by lymphopenia and heterophilia from 4 to 8 hours post LPS injection. Up to 48 hours post injection the leukopenia changed to a marked leukocytosis with longer-lasting monocytosis. Deficient dietary Arg elevated the total leukocyte counts compared with adequate and surplus dietary Arg. Furthermore, the stress indicator heterophil/lymphocyte ratio revealed a high-degreed stress response to LPS which was more pronounced in brown genotypes than in white ones. Additionally, deficient and surplus dietary Arg tended to intensify the LPS-induced stress response by increasing heterophil/lymphocyte ratio. Furthermore, LPS caused higher relative weights of the liver and spleen and decreased those of pancreas, bursa, thymus and cecal tonsils in turn. However, insufficient dietary Arg increased the relative weights of the examined liver and pancreas in pullets further.

Moreover, the haematological changes induced by LPS were accompanied by a genotype-specific fever response in cockerels and pullets. Whereas brown genotypes showed an initial hypothermia followed by a longer-lasting moderate hyperthermia, white genotypes exhibited a severe biphasic hyperthermia without initial hypothermia. Beside the fever response, within two hours LPS induced severe, unspecific sickness behaviour characterised by lethargy, anorexia, intensified respiration and ruffled feathers recovering until 12 hours post injection. Whereas high performing pullets exhibited sickness behaviour more pronounced than low performing pullets, dietary Arg did not modulate body temperature and behaviour.

Finally, within the first 24 hours post LPS injection both cockerels and pullets reduced their feed intake and lost body weight strongly. Thereby, low performing cockerels lost body weight to a lesser extent than their high performing counterparts and deficient dietary Arg intensified the loss of body weight in cockerels further. However, pullets showed neither a genotype-specific nor a dietary intensification of LPS-induced the loss of body weight. Additionally, the examination of pullets’ nitrogen metabolism and plasma amino acids
identified the loss of body weight as being a severe loss of body nitrogen. In the second 24 hours post LPS injection feed intake, weight gain and nitrogen retention recovered, but the analysis of plasma amino acids revealed a persistence of metabolic disorders at 48 hours post LPS injection. In LPS treated pullets the sum of plasma amino acids and the concentrations of Arg, L-citrulline, L-glutamate, L-methionine, L-ornithine, L-phenylalanine, L-proline, L-tryptophan and L-tyrosine were lower than in control birds. On the contrary, the plasma concentrations of L-aspartate, L-glutamine, L-lysine, 1-methyl-histidine and 3-methyl-histidine were increased by LPS. Besides, increasing concentrations of dietary Arg elevated plasma concentrations of Arg, L-citrulline, L-ornithine and 3-methyl-histidine subsequently.

In conclusion, the research conducted for this thesis confirms the indispensable character of dietary Arg for growth and performance in chickens, and emphasises the direct dependency of available plasma Arg on provided dietary Arg in general. Among the internal organs, the growth of the liver, pancreas and lymphoid organs thymus, spleen and bursa is most sensitive to dietary Arg. Furthermore, LPS triggers an acute-phase reaction with severe alterations in chicken’s behaviour, body temperature, differential blood counts and protein metabolism. The present research identifies deficient dietary Arg as a potential nutritional stress factor. Referring to the assumed diverse adaptability of high and low performing genotypes to varying dietary Arg under physiological and pathophysiological conditions, the present thesis indicates that the vast majority of examined parameters respond to Arg and LPS largely independent of chicken’s phylogeny and performance level. For that reason, further research is needed on the underlying mechanisms causing different Arg sensitivities of cells, organs and chicken strains under physiological and pathophysiological conditions.
ZUSAMMENFASSUNG

Marc-Alexander Lieboldt (2015)

Effekte diätetischen L-Arginins auf den Stoffwechsel und die Immunantwort in Legehühnern unterschiedlicher genetischer Herkünfte unter physiologischen und pathophysiologischen Bedingungen

Die Experimente der vorliegenden Doktorarbeit basierten auf einem Hühnermodell bestehend aus zwei weißen und zwei braunen Reinzuchtlegelinien jeweils mit hoher und niedriger
Legeleistung. Um die altersabhängigen Unterschiede im Nährstoffbedarf von Legehühnern angemessen zu berücksichtigen, wurden Küken (Schlupf bis Woche 7), Junghennen (Woche 8 bis 16) und Legehennen (Woche 16 bis 41) *ad libitum* mit Rationen gefüttert, die Arg jeweils zu 70, 100 und 200 % der altersspezifischen Versorgungsempfehlung enthielten. Um die Anpassungsfähigkeit der Legelinien an diätetisches Arg anhand ihrer Wachstums- und Leistungsantwort unter physiologischen Bedingungen zu prüfen, wurden 150 weibliche Eintagsküken je Linie gleichmäßig auf die drei Kükenrationen verteilt. Am Ende der Aufzuchtphase wurden 36 Junghennen pro Versuchsgruppe für die Beurteilung der Leistung während der Legeperiode weiter verwendet. Parallel zur Aufzuchtphase wurden zusätzlich 12 weibliche Eintagsküken pro Versuchsgruppe separat unter den gleichen Bedingungen aufgezogen, um das Genotyp-spezifische Organwachstum sowie organspezifische Empfindlichkeiten gegenüber diätetischem Arg in 6-Wochen-Abständen bis zur 18. Lebenswoche zu untersuchen.


Im Gegensatz zu den minderleistenden Genotypen wurden die hochleistenden Genotypen unbeabsichtigt und ungeachtet der Staffeln von diätetischem Arg mit Rohprotein während der Legeperiode unterversorgt. Folglich zeigten die hochleistenden Hennen einen starken Rückgang der Futteraufnahme und Legeleistung, gefolgt von einer schwerwiegenden Mobilisierung von Körpermasse. Hingegen erreichten die minderleistenden Genotypen ihr
genetisches Leistungspotential und reagierten auf den diätetischen Arg-Mangel mit einem Rückgang der Legeleistung, was die Annahme stützt, dass der Proteinbedarf gedeckt war.
Darüber hinaus waren die LPS-bedingten hämatologischen Veränderungen von einer Genotyp-spezifischen Fieberantwort in den Junghähnen und Junghennen begleitet. Während
braune Genotypen eine anfängliche Hypothermie gefolgt von einer länger andauernden moderaten Hyperthermie zeigten, wiesen die weißen Genotypen eine schwere biphasische Hyperthermie ohne anfängliche Hypothermie auf. Neben der Fieberantwort löste LPS binnen 2 Stunden ein deutliches, unspezifisches Krankheitsverhalten aus, das durch Lethargie, Anorexie, verstärkte Atmung sowie gesträubtes Gefieder geprägt war und sich bis 12 Stunden nach der Injektion wiederholte. Während die hochleistenden Junghennen deutlicheres Krankheitsverhalten zeigten als die minderleistenden Junghennen, beeinflusste diätetisches Arg weder die Körpertemperatur noch das Verhalten.


Zusammenfassend bestätigt die für die vorliegende Doktorarbeit durchgeführte Forschung die unverzichtbare Natur von diätetischem Arg für das Wachstum und die Leistung von Hühnern und betont allgemein die direkte Abhängigkeit des verfügbaren Plasma-Arg vom aufgenommenen diätetischen Arg. Von den inneren Organen ist das Wachstum der Leber, des Pankreas sowie das Wachstum der Lymphorgane Thymus, Milz und Bursa am
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ACKNOWLEDGEMENTS

I would like to sincerely thank everybody who has helped me during the implementation of the present thesis in all practical and academic matters. My special thanks go to:

Prof. Dr. Dr. Sven Dänicke, my principal supervisor and director of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Germany, for offering me the opportunity to be a paid member of his institute, supporting me with valuable discussions and comments as well as inspiring, professional supervision, and encouragement throughout my research during the past two and a half years.

Prof. Dr. Gerhard Breves, my Co-supervisor and director of the Institute of Physiology, University of Veterinary Medicine, Hannover, for his encouragement and acceptance to be my supervisor.

PD Dr. Ingrid Halle and Dr. Jana Frahm, Institute of Animal Nutrition, FLI, Federal Research Institute for Animal Health, Braunschweig, Germany, for their support, encouragement and active participation in my research.

Annerose Junghans, Institute of Animal Nutrition, FLI, Federal Research Institute for Animal Health, Braunschweig, Germany. Words cannot express my gratefulness for her excellent assistance in performing the experiments, her expertise in animal care and valuable and inspiring conversations during the experiments and beyond.

The members of the experimental research station of the Institute of Animal Welfare and Animal Husbandry, FLI, Federal Research Institute for Animal Health, Celle, Germany, for their support in performing the experiments, their excellent animal care and a pleasant working atmosphere.

The laboratory assistants of the working groups “Immuno-Nutrition” and “Basisanalytik” of the Institute of Animal Nutrition, FLI, Federal Research Institute for Animal Health, Braunschweig, Germany, for their excellent support in sample preparation and analysis.

All doctoral students and trainees of the Institute of Animal Nutrition, FLI, Federal Research Institute for Animal Health, Braunschweig, Germany, for their support in performing the experiments and creating a pleasant working atmosphere.

I especially would like to thank Nadine Schmidt and Melanie Schären, my office colleagues, for having a really good time, a lot of fun and a fantastic and pleasant working atmosphere.

Finally, I would like to deeply thank my family and little TONY for their endless love, constant support, excellent motivation no matter what happened and being there all the time.