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Resistance and tolerance to mixed nematode infections in chicken genotypes with extremely different growth rates

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ABSTRACT

Fast growing broilers are less able to cope with fitness related challenges. As the allocation of metabolic resources may be traded off between performance and defence functions in parasitized hosts, we hypothesized that fast growing broilers are more sensitive to mixed nematode infections compared with slower growing genotypes under the same environmental conditions. Therefore, we compared male birds of genotypes selected for either meat production (Ross-308, R) or egg production (Lohmann Brown Plus, LB) or for both purposes (Lohmann Dual, LD), to assess their resistance and tolerance to mixed nematode infections with *Ascaridia galli* and *Heterakis gallinarum*. While infections reduced feed intake in all three genotypes, feed conversion efficiency was not affected. Infections impaired growth performance only in R birds, indicating lower tolerance in the fast growing genotype compared with slower growing LB and LD genotypes. Impaired tolerance in R birds was associated with a relative nutrient scarcity due to an infection-induced lower feed intake. Resistance to experimentally induced infections depended on host genotype as well as on the worm species involved. Overall, the *A. galli* burden was higher in R than LB, whereas the burden of LD was not different from that of R and LB. In contrast, the *H. gallinarum* burden of first generation worms was similar in the three genotypes. Susceptibility to re-infection with *H. gallinarum* was higher in LB than in LD, whereas very low levels of re-infection were observed in R birds. Our data collectively suggest that resistance and tolerance to mixed nematode infections are sensitive to growth rate in chickens. These differences amongst genotypes may partly be associated with a mismatch between the actual nutrient supply and genotype-specific nutrient requirements.

1. Introduction

Infections with multiple nematode species are common in layer chickens (Kaufmann et al., 2011a; Thapa et al., 2015; Wuthijaree et al., 2017), and they occur not only in outdoor (Permin et al., 1999) but also in indoor systems (Grafl et al., 2017). Although nematode infections are rarely observed in broilers kept in intensive systems (Wilson et al., 1994; Kumar et al., 2015), slow growing broilers kept in organic systems may particularly be at risk due to the obligatory outdoor access and a prolonged fattening period that increases exposure to the infections. In line with the longer life expectancy, nematode infections can be highly prevalent (i.e. 89.9%) even in broiler breeders (Yazwinski et al., 2013). *Ascaridia galli* and *Heterakis gallinarum* are, globally, the two most prevalent nematode species parasitizing the chicken host (Abdelqader et al., 2008; Thapa et al., 2015; Wuthijaree et al., 2017). Infections with both ascarids are known to impair host performance (Dänicke et al., 2009; Daş et al., 2010, 2011, 2012; Schwarz et al., 2011a), and are associated with increased mortality (Hinrichsen et al., 2016). The adverse effects of ascarid infections on host performance are mediated, mainly through lower nutrient absorption (Hurwitz et al., 1972; Walker and Farrell, 1976; Schwarz et al., 2011b), impaired feed conversion efficiency and lower nutrient intake (Daş et al., 2010, 2011, 2012).

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Due to the strong genetic antagonism between reproduction and growth traits (Damme, 2015), modern chicken genotypes have been developed to efficiently produce either eggs or meat, but not simultaneously in the same animal. Although both one-way production modes target mainly protein deposition in the form of either eggs or meat, divergent genotypes with different life spans are used for each purpose. Genetic selection in broilers has increased the growth rate by 400% over 50 years (Zuidhof et al., 2014). There is, however, a large body of evidence that fast growing broilers have an impaired ability to cope with physiological, behavioural and immunological challenges (Koenen et al., 2002; Rauw, 2012) that are considered undesirable consequences of selection for increased production (Rauw et al., 1998). The impaired ability of broilers to cope with the fitness related challenges may be associated with allocation of finite resources in a given environment (Glazier, 2009), because selection for increased production (e.g. growth rate) will lead to a prioritized allocation of available resources toward production at the expense of other life history traits including immunity to pathogens (Rauw, 2012).

There is evidence for the existence of different types of immune programming in broiler and layer type chickens, which appears to be in line with their productive lifespan. The study of Koenen et al. (2002) showed that broilers have a lower cytokine response and are more specialized in mounting a strong short-term humoral immune response, whereas layers rely on a strong cellular response accompanied by a long-term humoral immune response. Indeed, a meta-analysis on the trade-off between growth and immune function in poultry concluded that genetic selection for rapid growth compromises immune function (van der Most et al., 2011). In line with this, Han and Smyth (1972) showed that fast growing broilers were more susceptible to Marek's disease than slow growing broilers. Furthermore, chicken genotypes that have been selected for different digestive efficiencies (Calenge et al., 2014) and performance traits (Han et al., 2016) differ in their susceptibility to bacterial infections. Compared with bacterial and viral infections, less is known about responses of chickens selected for increased growth to parasitic challenges. As in mammals, a chicken's immune system deals with intracellular (e.g. *Eimeria* spp.) and extracellular (e.g. nematodes) parasites through different immune responses, i.e. Th-1 and Th2 pathways, respectively (Degen et al., 2005). In a recent study Sakkas et al. (2018) showed that fast growing broilers were similar to slow growing broilers in terms of resistance and tolerance to coccidiosis when offered nutrient adequate diets (Sakkas et al., 2018). Resistance and tolerance to nematode infections have never been studied comparatively in chickens with different performance objectives that also greatly differ in growth rates. Genotype comparisons and heritability estimates indicate that there is considerable genetic variation in resistance to nematode infections in chickens (Permin and Ranvig, 2001; Gauly et al., 2002; Schou et al., 2003; Kaufmann et al., 2011b; Wongrak et al., 2015). These results are, however, from layer type chickens exposed to experimental or naturally occurring infections. There are no such detailed studies in broilers, and in general very little is known about their responses to nematode infections (Daş and Gauly, 2014; Ruhnke et al., 2017). As the allocation of metabolic resources may be traded off between performance and defence functions in the parasitized host (Coop and Kyriazakis, 1999; Colditz, 2008; van der Most et al., 2011), we hypothesized that fast growing genotypes are more vulnerable to nematode infections compared with slow growing genotypes under the same environmental conditions. Therefore, the aim of this study was to compare male birds of meat type, layer type and dual purpose chicken genotypes in terms of resistance and tolerance to mixed nematode infections. Growth performance, which is the most relevant performance parameter for rearing male birds of all chicken genotypes, served as the base for assessment of tolerance over the same sex.

2. Materials and methods

2.1. Birds, experimental design and ethics

A total of 668 male chicks of three divergent chicken genotypes, developed for either egg production (Lohmann Brown Plus, LB) or meat production (Ross-308, R), or for both purposes (Lohmann Dual, LD), were used. Ross-308 and LB birds were used as the positive and negative control genotypes for growth performance, respectively, whereas LD birds served as an additional genotype with an intermediate growth rate. The experiment was conducted twice in succession (temporal replication), and in each iteration, there were two infected and two uninfected groups of birds (spatial replication), which were kept in separate pens within the same experimental stable. In each spatial and temporal run, infected and uninfected control birds of all three genotypes were used simultaneously. An overview of the experimental design, replication blocks, animal numbers and sampling schedule over time within a run is given in Fig. 1. Birds of each genotype were either experimentally infected (n = 430) at an age of 1 week with a total of 500 infective eggs of *A. galli* and *H. gallinarum* in equal proportions to produce mixed nematode infections, or kept as uninfected controls (n = 238). From 2 weeks p.i. onwards, randomly selected infected and control birds from each genotype were necropsied at weekly intervals up to 9 weeks p.i. to quantify infection intensity with either nematode. Uninfected control birds were examined for accidental infections to exclude any potential confounding effects on performance and immune-related parameters. By 3 weeks p.i., individual faecal samples were collected from the birds 1 day prior to necropsy during captivity in individual cages to quantify nematode egg excretion at weekly intervals. All birds were individually wing tagged on the day of infection to ensure the collection of individual data over time. Individual body weight and pen-based feed consumption (n = 4 per genotype and infection status) were determined at weekly intervals. Blood samples were collected weekly during necropsy starting at 2 weeks p.i.

The experiment was conducted in accordance with animal welfare rules (animal care and handling, stunning, necropsies) and was approved by the State ethics committee for animal experimentation (Mecklenburg-Western Pomerania State Office for Agriculture, Food Safety and Fishery, Germany; permission no: AZ: 7221.3-1-066/15). The experimental infection procedures were in line with the relevant guidelines of the World Association for the Advancement of Veterinary Parasitology for poultry (Yazwinski et al., 2003).

2.2. Housing and management

The pens containing infected and uninfected animals were in two separate rooms of an experimental research facility for poultry to avoid cross-contamination between the infected and uninfected groups of birds (Fig. 1). The birds were reared in a floor husbandry system on wood shavings as litter material. Litter was not removed during the infection period, to allow re-infections to occur. Additional litter, corrected for total body weight per m^2, was added to all pens at the same time to ensure similar litter conditions for all genotypes in different pens. The climatic conditions were fully controlled by an automatic system to ensure uniform temperature, light and aeration conditions across the pens within and between rooms. The temperature was gradually decreased from 34 °C on the first day to close to 20 °C in the 10th week of life. Feed and

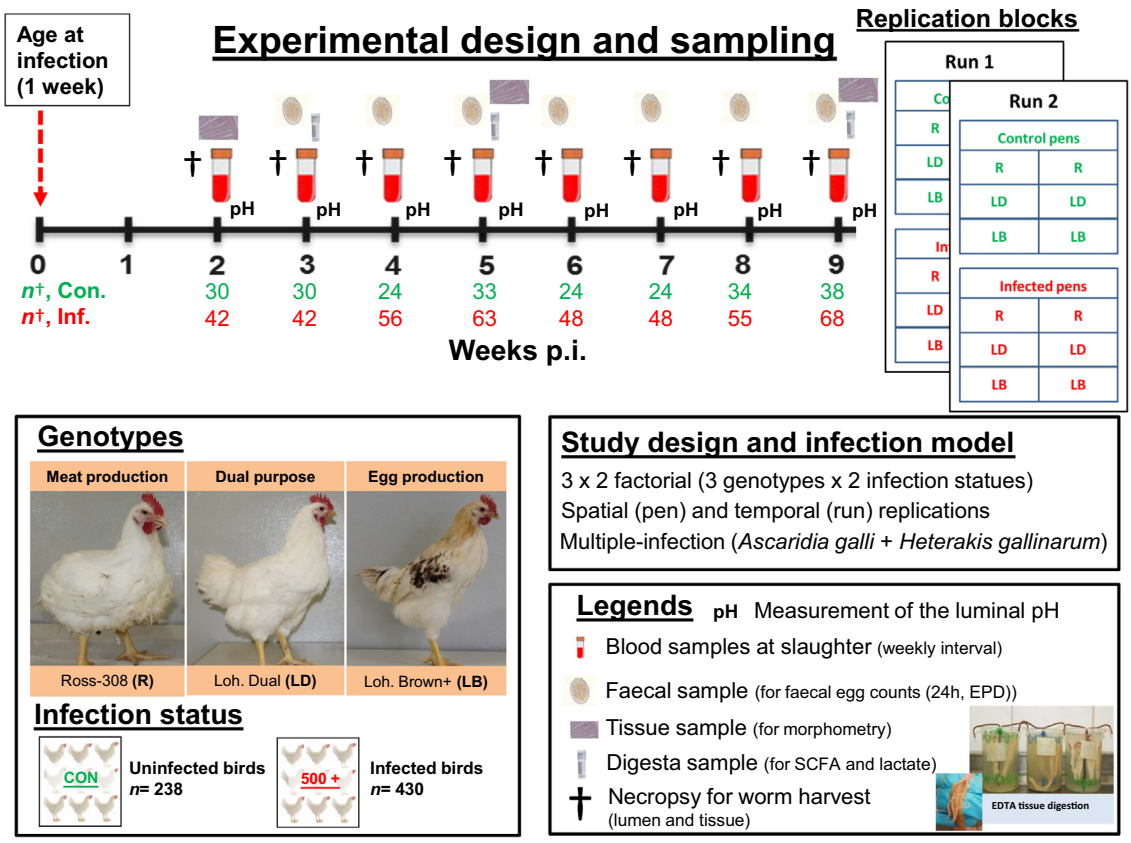

Fig. 1. Summary of the experimental design and sampling schedules. Con, control; Inf, infected; LB, Lohmann Brown Plus; LD, Lohmann Dual; R, Ross-308; SCFA, short chain fatty acid; $n^{\dagger}$, number of necropsied birds.

water were given ad libitum. All genotypes were fed on the same starter (day 1–14; 12.6 MJ of metabolizable energy (ME) and 219 g of crude protein (CP) per kg of feed), grower (day 14–53; 13.0 MJ of ME and 204 g of CP per kg of feed) and finisher diets (day 53–70; 13.4 MJ of ME and 185 g of CP per kg of feed), with a transition phase of approximately 3 days between the different diets. The diets provided or exceeded age-specific nutrient requirements of the broiler birds (Aviagen, 2014; http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross308BroilerNutritionSpecs2014-EN.pdf). The detailed composition and nutrient contents of the diets are given elsewhere (Urban et al., 2018). Animal density for each genotype did not exceed 25 kg/m^2 at any time point, ensuring that standards for an ecological production system regarding the space requirements of broilers were met. The birds received no vaccinations or medical treatment, including anthelmintics, before or after infection. Mortality was recorded throughout the experimental period.

2.3. Experimental infection

The infection material was derived from the intestines of naturally infected chickens (i.e., free-range chickens) that were collected from several slaughterhouses and farms across northern Germany. The preparation techniques and incubation conditions for the infection material have previously been described by Stehr et al. (2018). On the day of infection (7 days of age), the incubation media of both *A. galli* and *H. gallinarum* were separately filtered through a sieve (36 μm mesh size), which was followed by rinsing to collect the washed eggs in saline solution (NaCl, 0.9%). Based on morphological classification (Rahimian et al., 2016), only fully embryonated eggs, which are considered infectious, were counted to determine the percentage of embryonated eggs per ml of suspension. The single infection dose for each worm species was adjusted to 250 embryonated eggs ($SD_{A.\ galli}$ = 23.7; $SD_{H.\ gallinarum}$ = 12.4) per 0.1 ml of NaCl (0.9%), which was administered to each bird in a final inoculum of 0.2 ml of NaCl, containing a total of 500 eggs with equal proportions of the two worm species. The infection dose was administered orally using a 5 cm oesophageal cannula as described previously (Daş et al., 2010), whereas uninfected control birds received a sham oral treatment with the same amount (0.2 ml) of NaCl solution.

2.4. Worm harvest

Quantification of the worm burden was performed weekly from 2 to 9 weeks p.i. Before necropsy, the birds were fasted for 3 h to allow standardized partial emptying of the intestine. Immediately post mortem, the gastrointestinal tract was removed, and the caecum and small intestine (SI) were separated. The SI was divided into the jejunum and ileum at the Meckel's diverticulum (Svihus, 2014). The duodenum was excluded from quantification, as macroscopic examinations indicated that this intestinal section is not the normal habitat for *A. galli*. The jejunum and ileum were opened longitudinally, and the intestinal contents, separated by section, were washed separately through sieves with mesh sizes of 36 μm and 100 μm at 2–5 weeks p.i. and 6–9 weeks p.i., respectively. The quantification of tissue-associated *A. galli* larvae was restricted to the jejunal section, which is the main preferred site of larval stages (Ferdushy et al., 2013). The procedure for larval recovery by using the EDTA-incubation method was recently described in Stehr et al. (2018).

Heterakis gallinarum worms were harvested from the lumen contents by rinsing the opened caeca in sieves (mesh sizes 20–36 μm). Both *A. galli* and *H. gallinarum* worms collected from individual birds were then separately transferred to Petri dishes for counting, sex differentiation and length measurements. Uninfected birds were also examined for the presence of worms in the SI (tissue and lumen) and caecum to exclude accidental infections with either nematode.

2.5. Worm population structure

The initial classification of the harvested worms into larvae, females and males was based on morphological characteristics. For both worm species, differentiation between male and female worms can be easily performed according to the presence of spicules. While there is no difficulty in distinguishing mature and immature females of *H. gallinarum* based on the presence of eggs in the uteri, the presence/absence of eggs in the uterus of intact *A. galli* females cannot be definitively confirmed. Therefore, we used a length cut-off (immature $\leq$ 43.5 mm > mature) that allowed precise separation between female worms (Stehr et al., 2018). The classified worms (larvae, immature females, mature females and males) were then individually measured to determine the worm length for each worm stage and sex. For this purpose, only intact worms (maximum of 10 per bird) from each developmental stage and sex were randomly selected and measured to determine lengths on a ruler under a stereo-microscope. The length dataset was used to calculate a new parameter, overall average worm length (OWL), combining worm length across different worm stages from each bird. Therefore, the average length (y) of each specific worm stage was multiplied by the corresponding number of worms in each developmental stage (n) to adjust their respective proportions in the total worm burden (N) as described below.

$$\mathrm{OWL} = [(\mathrm{larvae}, \mathrm{y_x n}) + (\mathrm{immature}, \mathrm{y_x n}) + (\mathrm{mature}, \mathrm{y_x n}) + (\mathrm{males}, \mathrm{y_x n})/\mathrm{N}]$$

All *Heterakis* worms up to 4 weeks p.i. were considered to originate from the experimental infection and were therefore defined as first generation worms. By 5 weeks p.i., the number of first generation worms was calculated as the total worm burden minus the number of larvae and immature worms, as these juvenile worms must have originated from reinfection. Non-larval worms were defined as the total worm burden minus larvae and included mature and immature worms that were sexually differentiable by morphological characteristics.

2.6. Faecal egg counts

To quantify the nematode egg concentration in faeces (eggs per gram faeces, EPG), the daily total faeces were thoroughly homogenized. A random sub-sample (4 g) of homogenized faeces was then analysed with a modified version of the McMaster egg counting technique (MAFF, 1986). A saturated NaCl solution (density $\geq$ 1.2 g/ml) was used as the flotation liquid. The minimum detection level of the egg counting technique was 50 nematode eggs/g of faeces. By multiplying the amount of daily excreted faeces with EPG, the number of eggs excreted within 24 h (eggs per day, EPD) was then estimated (n = 320 samples). Eggs of *A. galli* and *H. gallinarum* were counted together because they could not be reliably differentiated (Kaufmann, 1996).

2.7. ELISA for ascarid-specific antibodies

An ELISA (Daş et al., 2017) was used to quantify anti-ascarid-specific IgY levels in EDTA-plasma samples collected during weekly necropsies (2–9 weeks p.i.; n = 646). Plasma was obtained from the blood samples by centrifugation at 2500*g* for 20 min and 4 °C, and then stored at −20 °C. The laboratory-specific intra-assay coefficient of variability (CV) and inter-assay CV for this analysis were 5.0% and 8.4%, respectively.

2.8. ELISA for immunoglobulins (Ig) IgY, IgM and IgA

Commercial ELISA Kits (IgY: Kit No. E30-104; IgM: Kit No. E30-103; IgA: Kit No. E30-102; Bethyl Laboratories, Inc, Montgomery, TX, USA) were used to analyse immunoglobulin concentrations (IgY, IgM, IgA) in EDTA-plasma samples at 2, 5 and 9 weeks p.i. (n = 273). The sample collection and preparation methods were identical to those described for the ascarid-specific antibodies. ELISA was performed according to the manufacturer's instructions. A pooled plasma sample served as a control among all plates. The laboratory-specific intra-assay CV and inter-assay CV for the analysis ranged between 5.0–7.6% and 7.7–10.4%, respectively.

2.9. Luminal pH, short-chain fatty acids (SCFAs) and lactate (LA) in the intestines

The luminal pH of the SI and caecum was determined individually at weekly intervals (2–9 weeks p.i.) in both infected and non-infected birds at necropsy. Within 10 min post mortem, the luminal pH was measured in duplicate using a glass pH electrode directly inserted into the digesta (Spear tip, VWR GmbH, Darmstadt, Germany). For the SI, the pH was determined on both the proximal (jejunum) and distal (ileum) sites of Meckel's diverticulum. The caecal pH was measured twice at the blind end of the left caecum. The duplicate pH measurements correlated well (r = 0.94, P < 0.001) for both the SI and caecum, and an individual location-specific mean pH value was used for statistical analyses.

SCFA and LA concentrations in both the ileal and caecal digesta were determined at the Department of Veterinary Medicine, Freie Universität Berlin, Germany. As the whole intestinal contents were used for exact quantification of the worm burden, digesta samples for SCFA and LA analyses were obtained from counterpart birds from the same study group. All the birds used in these study analyses were identically treated/infected, housed and necropsied on the same day. Digesta samples were collected from 18 fed birds (i.e., three birds per genotype and infection status) at the necropsy time-points of 3, 5 and 9 weeks p.i. (n = 108). Immediately post mortem, samples from the ileum and left caecum were transferred to cryo-tubes, snap frozen in liquid nitrogen and stored at −20 °C for subsequent analysis. SCFA and LA concentrations in the ileal and caecal digesta were determined via gas chromatography and HPLC as described previously (Goodarzi Boroojeni et al., 2014).

2.10. Intestinal size and histomorphometry

The length and weight of the SI and caeca were measured at 0 and 9 weeks p.i. (i.e., at an age of 7 and 63 days, respectively). For light microscopy and morphometry, the intestinal tissue (1 cm^2) from the jejunum was fixed in a 4% neutral formaldehyde solution. After rinsing in water, the tissue samples (n = 54, collected at 2, 5 and 9 weeks p.i.) were dehydrated in a graded series of ethanol (30%, 50%, 70%, 90% and absolute ethanol), cleared in benzene and then saturated with, and embedded in, paraffin. Sections of 5 μm thickness (10 slices of each sample) were stained with H&E and observed under a light microscope as described previously (Zitnan et al., 2008). The heights and widths of 30 villi and the depths of 30 crypts were determined with the computer-operated Image C image analysis system (Imtronic GmbH, Berlin, Germany) and the IMES (interactive measurement) analysis programme, using a colour video camera (SONY 3 CCD, Sony

Electronics Ltd., Tokyo, Japan) and a light microscope (Axiolab, Carl Zeiss AG, Jena, Germany).

2.11. Statistical analyses

Nematode-free control birds were excluded from the analyses of the worm burden and faecal egg count (FEC) data. Worm burden, FEC and antibody data were analysed following log transformation [Ln (y + 1)] to correct for heterogeneity of variance and produce approximately normally distributed data. All variables were then subjected to ANOVA by using the MIXED procedure in the SAS/STAT (Version 9.4) software of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA). The statistical model for all worm-related parameters (worm burden, FEC, worm length) included the fixed effects of genotype, weeks p.i. and their interaction, plus pen and run effects. As individual performance (body weight, average daily gain) as well as antibody data were available for the uninfected control birds, the model included the effects of infections, genotype, week p.i. and their interactions, plus run and pen effects. The effect of repeated sampling of the birds (subject) over weeks was considered with the REPEATED statement of the MIXED procedure, and the structure of the block diagonal residual covariance matrix was set to AR(1), as this setting provided the best fit of the parameters (e.g., smallest Akaike Information Criterion) for the fitted models. For pen-based data (average daily feed intake, feed conversion ratio), the model was the same as for the individual performance data, but the pen effect was omitted because it was considered as the repeatedly measured experimental unit. To account for the decreasing number of birds in each pen over time (due to necropsies), the model for pen-based data included the WEIGHT statement. The covariance matrix for this model was also AR(1).

Least-squares means (LSM) and their standard errors (SE) were computed for each fixed effect in the model, and all pairwise differences in these LSMs were tested with the Tukey–Kramer test, a procedure for pairwise multiple comparisons. In addition, the SLICE statement of the MIXED procedure was used for performing partitioned analyses of the LSMs for the two- or three-way interactions (e.g., test of infections within the levels of week p.i. in each genotype). Effects and differences were considered significant at $P < 0.05$. Overall cumulative mortality was analysed with the Chi-square test by performing multiple comparisons between infected and uninfected birds as well as between genotypes.

3. Results

3.1. Host performance

In comparison with R birds (2.8%), overall mortality during the infection period was lower ($P = 0.012$) in LD (0.3%) and tended ($P = 0.09$) to be lower in LB birds (0.9%). Infections did not influence ($P = 0.763$) mortality in any genotype (i.e., 1.2% and 1.5% for control and infected birds, respectively). Body weight (BW) increased steadily over the weeks of the experiment ($P < 0.001$), and differed significantly among the genotypes in the order of R > LD > LB ($P < 0.001$; Table 1). A significant interaction among infection, genotype and time effects ($P < 0.001$; Fig. 2A) indicated a lower BW in infected R birds compared with uninfected R controls by 3 weeks p.i., which was not observed for LD or LB birds ($P > 0.05$). Similar to BW, the overall average daily gain (ADG) was significantly different among the three genotypes, in the order of R > LD > LB ($P < 0.001$). The ADG was temporarily affected in the infected R birds ($P < 0.05$) at the time points of 3, 4, 5 and 8 weeks p.i. (Fig. 2B). No significant effect of infections on the ADG was found in LB or LD ($P > 0.05$). The overall average daily feed intake (ADFI) was different among the genotypes in the order of R > LD > LB ($P < 0.001$). The difference in the feed intake of the genotypes was fairly constant over the weeks of the experiment

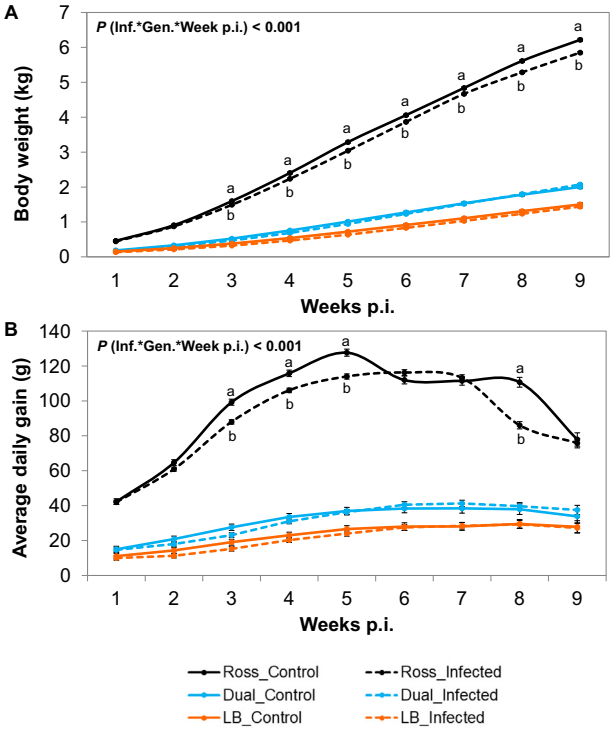

Fig. 2. Time-dependent effects of mixed nematode infections on body weight (A) and average daily weight gain (B) in divergent chicken genotypes. a, b indicate significant ($P < 0.05$) differences between infected and un-infected control birds of the same genotype at the same time point. The values are LSMEANS with SE on the error bars. (Number of birds = 668; sample size $n = 3496$). LB, Lohmann Brown Plus; Ross, Ross-308.

Table 1
Growth performance, feed intake and feed conversion efficiency in divergent chicken genotypes exposed to mixed nematode infections.

	Main effects of genotype					Infection effect				Interaction, $P \leq$	
Item	R	LD	LB	SE	$P \leq$	Con.	Inf.	SE	$P \leq$	Inf.*wpi	Inf.*Gen*wpi
Body weight (g)	2876[a]	940[b]	667[c]	17.12	0.001	1533	1456	16.33	0.001	0.001	0.001
Daily weight gain (g)	85.2[a]	29.1[b]	20.7[c]	0.62	0.001	46.2	43.8	0.56	0.001	0.002	0.001
Feed intake (g/d)	178.1[a]	79.8[b]	57.6[c]	2.13	0.001	107.9	102.5	1.90	0.040	0.231	0.905
FCR (g/g)	1.92[a]	2.42[b]	2.43[b]	0.05	0.001	2.25	2.27	0.04	0.767	0.661	0.773

[a,b,c]Genotypes shown with different letters differ significantly (Tukey, $P < 0.05$).
Analyses for body weight (sample size $n = 3496$ observations) and average daily weight gain (sample size $n = 3493$) are based on repeated individual bird data. Feed intake and FCR are based on repeated pen data. The number of pens used per week was four per genotype and infection group (sample size $n = 216$).
FCR, feed conversion ratio (feed intake/weight gain); R, Ross-308; LD, Lohmann Dual; LB, Lohmann Brown Plus; Con, uninfected controls; Inf., infected; Gen, genotype; wpi, weeks p.i.; g/d, gram per day.

($P = 0.231$; Table 1). Infected birds exhibited a significantly lower ADFI ($P = 0.040$) than the controls, without an interaction with genotype or time ($P > 0.05$). The overall feed conversion ratio (FCR) was not different between LD and LB ($P = 0.996$), but the FCR of these two genotypes was significantly higher than that of R ($P < 0.001$). Infections did not affect the overall FCR ($P = 0.767$; Table 1).

3.2. FEC

The faecal samples of all genotypes were egg-negative at 3 weeks p.i. The first egg-positive samples were encountered at 4 weeks p.i. in LD and LB birds, and at 6 weeks p.i. in R birds. The three genotypes had similar EPG and EPD levels ($P > 0.05$; Table 2). Although the FECs increased over the experimental weeks ($P = 0.005$), there was no significant difference among the genotypes at any time point ($P > 0.05$).

3.3. Worm burden

The overall average *A. galli* counts (total burden) were significantly higher in R compared with LB ($P = 0.049$), while the average *A. galli* burden of LD was not different from those of the other two genotypes ($P > 0.05$; Table 2). The genotypes did not differ in terms of *A. galli* larval counts ($P = 0.137$), whereas adult worm counts differed among the genotypes in the order of R > LD > LB ($P < 0.001$; Table 2). The overall average burden across all genotypes decreased ($P < 0.001$) over time from 95 ± 4.0 (LSM ± SE) worms/bird at 2 weeks p.i. to 3 ± 3.2 worms/bird at 9 weeks p.i. Similarly, larval counts decreased continuously over the weeks of the experiment ($P < 0.001$), and no re-infection occurred. Non-larval worm counts were higher in R than in LD and LB at the early phase of infection (3–5 weeks p.i.; data not shown), while the differences became much smaller in the subsequent weeks.

The total number of tissue larvae in the jejunum did not differ among the genotypes ($P = 0.331$). Although genotype effects did not interact with time ($P = 0.101$), a three-fold higher average number of larvae was recovered from the tissue of R birds at 2 weeks p.i. than from the other two genotypes (data not shown). The percentage of tissue larvae within the total burden was significantly higher ($P < 0.001$) in R than in LD and LB (Table 2) over the whole experimental period ($P = 0.555$). Similar to the lumen larvae, there was a significant decrease in the number of tissue larvae over time ($P < 0.001$; Fig. 3A). A significantly greater number of larvae was detected in the lumen than in the jejunum tissue ($P = 0.001$).

There was a genotype-dependent worm distribution between the jejunum and ileum. As shown in Fig. 3B, the percentage of lumen larvae was similar ($P > 0.05$) between the two locations in R, whereas a higher percentage of larvae was recovered from the jejunum than the ileum in both LD and LB ($P < 0.05$). Similar to larval stages, a higher percentage of the total worm burden was found in the jejunum than in the ileum ($P = 0.001$). Although the jejunal worm burden was not significantly different among the genotypes ($P > 0.05$), a higher percentage of worms was recovered from the ileum of R birds than from those of LD and LB birds ($P < 0.05$; Fig. 3B).

The overall average total *H. gallinarum* burden was different among the three genotypes ($P = 0.031$; Table 2). The LB had a higher number of *H. gallinarum* than R ($P = 0.034$), while the burden of LD was not different from those of LB or R ($P > 0.05$; Table 2). The numbers of first generation *H. gallinarum* worms, originating from the experimental infection, did not differ among the genotypes ($P = 0.449$; Table 2). Similarly, the numbers of first generation larvae did not differ ($P = 0.887$), whereas the numbers of larvae resulting from re-infection significantly differed among the genotypes in the order of LB > LD > R ($P < 0.001$) (Table 2). The overall average *H. gallinarum* burden across the three genotypes decreased from 2 to 7 weeks p.i. and increased thereafter. Almost no re-infection occurred in R birds, whereas LD and, particularly, LB birds harboured increasing numbers of larvae starting at 7 weeks p.i. (Fig. 4). At 9 weeks p.i., the highest number of *H. gallinarum* larvae was found in LB, followed by LD and R, with significant ($P < 0.05$) differences among all three genotypes.

The OWL of *A. galli* was significantly ($P < 0.001$; Table 2) higher in R and LD than in LB, with no interaction over time ($P = 0.172$). In contrast, the OWL of *H. gallinarum* was significantly higher

Table 2
Overall effects of host genotype on faecal egg counts (FEC), worm burdens and worm length in divergent chicken genotypes exposed to mixed-nematode infections.

	Host genotype				$P \leq$		
Item	R	LD	LB	SE	Gen.	wpi	Gen.*wpi
FEC							
EPG (n/bird)	19	20	15	5.8	0.884	0.005	0.856
EPD (n/bird)	2172	1288	556	529.7	0.953	0.005	0.820
Ascaridia galli							
Total burden (n/bird)	26.9[a]	25.2[ab]	24.3[b]	2.35	0.049	0.001	0.123
Total larva (n/bird)	21.1	22.1	23.1	2.29	0.137	0.001	0.514
Non-larva worms[e] (n/bird)	5.6[a]	3.0[b]	1.3[c]	0.07	0.001	0.001	0.001
Lumen larva (n/bird)	17.7[d]	20.0	21.1[d]	2.04	0.075	0.001	0.568
Tissue larva (n/bird)	3.4	2.1	2.1	0.49	0.331	0.001	0.101
Tissue larva (%)	42.5[a]	22.4[b]	27.5[b]	3.57	0.001	0.001	0.555
OWL (mm)	16.3[a]	15.0[a]	11.3[b]	0.922	0.001	0.001	0.172
Heterakis gallinarum							
Total burden (n/bird)	8.0[b]	9.1[ab]	11.7[a]	1.17	0.031	0.001	0.201
First generation (n/bird)	7.9	8.0	9.4	1.10	0.449	0.001	0.662
Total larva (n/bird)	1.5[b]	2.8[a]	4.4[a]	0.66	0.001	0.001	0.001
Larva 1st gen. (n/bird)	3.5	4.6	5.7	2.73	0.887	0.001	0.631
Larva 2nd gen. (n/bird)	0.2[c]	1.8[b]	3.6[a]	0.39	0.001	0.001	0.001
OWL (mm)	6.49	6.43	6.19	0.213	0.531	0.001	0.002

[a,b,c]Genotypes shown with different letters differ significantly (Tukey, $P < 0.05$).
[d]Genotypes tend to differ (Tukey, $P < 0.10$).
Faecal samples were collected from 3 to 9 weeks p.i. (sample size $n = 321$). Sample size for worm burden data, $n = 422$. Analysis of overall worm length (OWL) included 304 and 378 average length measurements weighed for developmental stages of *Ascaridia galli* and *Heterakis gallinarum*, respectively.
[e]Non-larval worms, defined as the worm burden minus larvae and includes mature and immature worms that are sexually differentiable by morphological characteristics.
EPG, number of eggs per gram faeces; EPD, number of eggs excreted within 24 h; R, Ross-308; LD, Lohmann Dual; LB, Lohmann Brown Plus; Con, uninfected controls; Inf., infected; Gen, genotype; wpi, weeks p.i. Larva 1st gen./2nd gen., larval stages of the first and generation worms descending from experimental and naturally occurring re-infections, respectively.

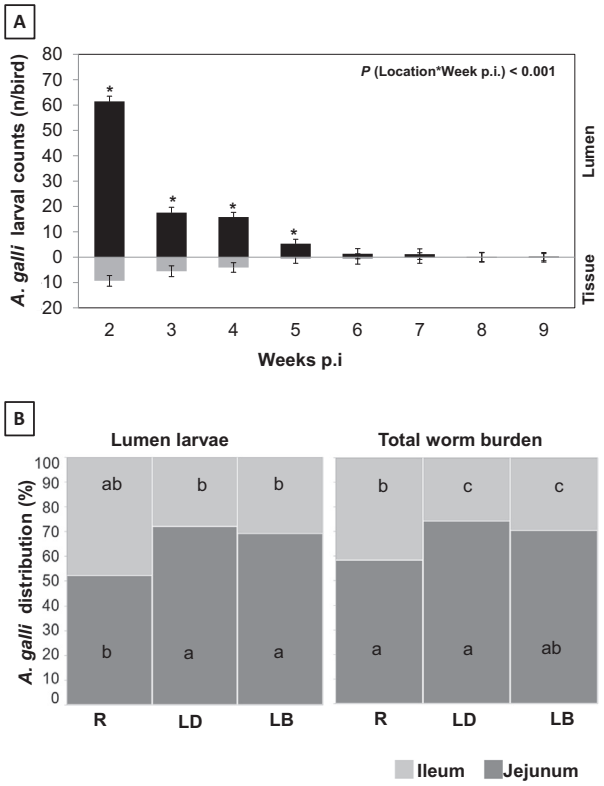

Fig. 3. Distribution of *Ascaridia galli* larvae between tissue wall and lumen in jejunum over time (A), and the localisation of worms between jejunum and ileum in different chicken host genotypes (B). (A) Asterisk (*) indicates a significant (Tukey, $P < 0.05$) difference between the number of larvae in the lumen and the tissue wall (sample size $n = 842$). (B) Values shown with different letters (a,b,c) differ significantly (Tukey, $P < 0.05$) (sample size $n = 843$). LB, Lohmann Brown Plus; LD, Lohmann Dual; R, Ross-308.

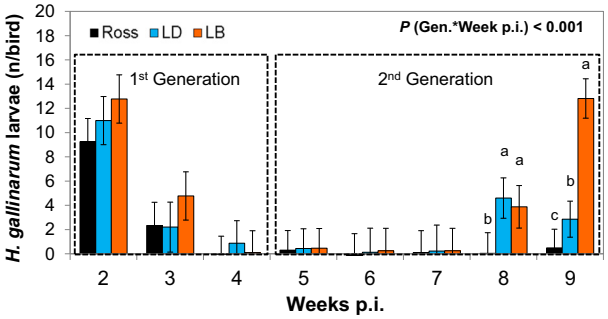

Fig. 4. *Heterakis gallinarum* larvae in divergent chicken host genotypes after experimentally-induced and naturally occurring (re)-infections. Host genotypes sharing no common letters (a–c) at a given time point differ significantly (Tukey, $P < 0.05$). LSMEANS and SE represent untransformed data; *P*-values are based on the transformed data (sample size $n = 422$; total number of birds necropsied at each week p.i. ranged from 42 to 68). LB, Lohmann Brown Plus; LD, Lohmann Dual; Ross, Ross-308.

($P < 0.05$) in LD than in LB only at 8 weeks p.i. (Supplementary Fig. S1). At 9 weeks p.i., the OWL in both R and LD was higher compared with that of LB ($P < 0.05$), and the OWL of R tended to be higher than that of LD ($P = 0.076$).

3.4. Ascarid-specific IgY antibodies

Overall, average plasma ascarid-specific IgY levels tended to be higher in LB than in R ($P = 0.062$; Table 3). Independent of host genotype, infections significantly increased IgY levels ($P < 0.001$). With the exception of the time point 3 weeks p.i., infected birds exhibited significantly higher ascarid-specific IgY levels than their uninfected counterparts during the study period (i.e., from 2 to 9 weeks p.i.; Fig. 5).

3.5. Immunoglobulins

The overall IgY concentration in plasma was higher in LD and LB than in R ($P = 0.002$; Table 3). However this effect was dependent on time after infection ($P = 0.003$), with LD and LB exhibiting higher concentrations than R at 2 weeks p.i. (Supplementary Fig. S2A). At 5 weeks p.i., the IgY concentration was higher in LD than in R ($P < 0.05$), whereas LB did not differ from the two other genotypes ($P > 0.05$). No differences were found among the genotypes at 9 week p.i. ($P > 0.05$). Although infections increased the overall IgY concentration (P = 0.002; Table 3), this effect was only present in R and LD birds ($P = 0.048$, Supplementary Fig. S2B). Overall IgM concentrations were higher in R than in LD and LB ($P = 0.001$; Table 3), particularly at 9 weeks p.i. (Supplementary Fig. S2C). Independent of host genotype ($P = 0.546$), infected birds had higher ($P < 0.001$) IgM concentrations than their uninfected counterparts (Table 3), due to a temporary increase at 2 weeks p.i. ($P < 0.001$; Supplementary Fig. S3D). IgA concentrations did not differ among the genotypes ($P = 0.227$; Table 3), whilst infections increased IgA concentrations in all genotypes at 2 weeks p.i. ($P = 0.002$, Supplementary Fig. S2E).

3.6. Intestinal size and macroscopic alterations in the caecum

At 1 week of age (0 weeks p.i., infection day), the lengths and weights of the SI were significantly higher in R than in the other genotypes ($P < 0.001$), with LD and LB showing similar weights ($P > 0.05$; Table 4). The caeca were longer in R than in LB ($P < 0.05$) and tended to be longer than in LD ($P = 0.069$), whereas caecal length was not different between LD and LB ($P > 0.05$). The total full weight of the caeca differed among the genotypes in the order of R > LD > LB ($P < 0.001$; Table 4). Similar to 0 weeks p.i., at 10 weeks of age (i.e. 9 weeks p.i.), the SI was larger in R than in LD and LB ($P < 0.001$), with no difference between the latter two genotypes ($P > 0.05$). The weight of the SI differed in the order of R > LD > LB ($P = 0.001$; Table 4). SI length was not affected by infection at 9 weeks p.i. ($P = 0.851$). Caecum length was similar between LD and LB ($P > 0.10$), whereas the caeca of R birds were larger ($P < 0.001$). Full caecum weight was in the order of R > LD > LB ($P < 0.001$). Infected birds exhibited shorter caeca than did uninfected controls at 9 weeks p.i. ($P < 0.001$), although caecal weight was not influenced by the infection ($P = 0.160$). The worm burden of both species did not correlate with the length or weight of their predilection sites ($P > 0.05$). Macroscopic alterations (e.g., mucosal bleeding, thickened caecal wall and fibrinous content) in the caeca were observed in 13.1%, 18.9% and 19.2% of the infected birds in the R, LD and LB genotypes, respectively. The percentage of macroscopically affected caeca decreased across the weeks of infection in all three genotypes (from 45.2 % at 2 weeks p.i. to 4.4% at 9 weeks p.i.).

3.7. Intestinal morphometry

Villus height (VH; $P < 0.001$, Table 5) and the ratio of villus height to crypt depth ($P = 0.005$) were significantly different among the genotypes, with R exhibiting higher ($P < 0.05$) values than LD and LB. Villus width was greater for R and LD than for LB ($P = 0.001$). Crypt depth was not different among the genotypes ($P = 0.653$). An interaction between genotype and week p.i. ($P = 0.002$) indicated that the differences in VH among the

Table 3
Infection-specific and unspecific humoral immune responses in relation to host genotype and mixed nematode infections in divergent chicken genotypes.

	Main effects of genotypes					Infection effect					Interaction, *P* values, ≤			
Item	R	LD	LB	SE	P,≤	Con.	Inf.	SE	P,≤	wpi	Inf.*Gen.	Inf.*wpi	Gen.*wpi	Inf.*Gen.*wpi
Asc-IgY[d]	8.68[c]	10.02	11.35[c]	1.45	0.062	6.11	13.92	1.30	0.001	0.001	0.894	0.001	0.079	0.061
IgY[e]	3.10[b]	3.87[a]	3.43[a]	0.24	0.002	2.82	4.12	0.22	0.001	0.001	0.048	0.062	0.003	0.275
IgM[e]	0.30[a]	0.18[b]	0.21[b]	0.02	0.001	0.21	0.26	0.01	0.001	0.001	0.546	0.001	0.001	0.661
IgA[e]	0.25	0.25	0.24	0.02	0.277	0.23	0.26	0.02	0.995	0.250	0.526	0.002	0.670	0.478

[a,b]Genotypes with different letters differ significantly (Tukey, $P < 0.05$). [c]Genotypes tend to differ (Tukey, $P < 0.10$).
Measurement units: [d]mU/ml, [e]mg/ml. Plasma samples for ascarid-specific IgY were taken weekly from 2 to 9 weeks p.i. (wpi) ($n = 646$). The samples for IgY, IgM, IgA were taken at 2, 5 and 9 wpi ($n = 271$).
Asc. IgY, ascarid-specific IgY; Ig, Immunoglobulin; R, Ross-308; LD, Lohmann Dual; LB, Lohmann Brown Plus; Con, uninfected controls; Inf., infected; Gen, genotype.

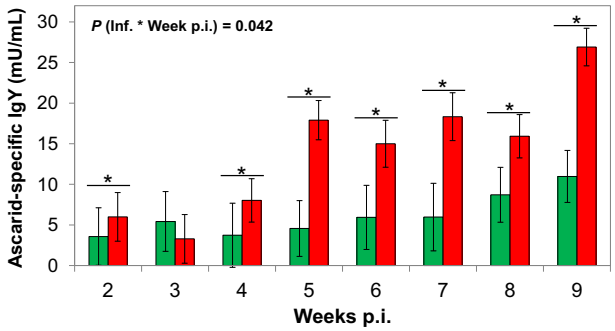

Fig. 5. Course of ascarid-specific IgY development in infected (● (black)) and uninfected (● (grey)) control chickens. An asterisk (*) indicates a significant (Tukey, $P < 0.05$) difference between infected and uninfected birds at the given time points. LSMEANS and SE represent untransformed data; *P* values are based on the transformed data (sample size $n = 646$ plasma samples ($n = 72$–96 birds/week p.i.)).

genotypes were time-dependent. At 2 weeks p.i., VH followed the order of R > LD > LB, whereas at 5 weeks p.i., the VH of R was significantly higher ($P < 0.05$) than those of LD and LB. At 9 weeks p.i., the genotypes did not differ for VH ($P > 0.05$). Villus width differed among the genotypes only at 9 weeks p.i., with LB exhibiting a narrower villus width than R and LD ($P < 0.05$). Villus width and crypt depth were not influenced by infection ($P > 0.05$; Table 5). A significant interaction among the effects of infection, genotype and week p.i. ($P = 0.013$), however, indicated a tendency ($P < 0.10$; Supplementary Fig. S3) towards a shorter VH in the infected LD birds at 2 and 9 weeks p.i. compared with the corresponding uninfected controls. The VH of R and LB was not affected by infection ($P > 0.05$). Additionally, the VH:crypt depth ratio was significantly lower in infected birds ($P = 0.048$).

3.8. Luminal pH in the small intestine (SI) and caecum

Luminal pH in SI was higher in R than in LB at 7 and 8 weeks p.i., whereas LD did not differ from the other two genotypes ($P = 0.007$; Fig. 6A). Infection lowered the luminal pH in the SI ($P < 0.001$; Table 6) independent of any interactions with host genotype ($P = 0.627$) and week p.i. ($P = 0.234$). The intra-caecal pH was different among the genotypes in the order of R > LB > LD ($P = 0.001$; Table 6). An interaction between genotype and time ($P < 0.001$) indicated significant alterations in caecal pH among the genotypes by time (Fig. 6B). Infection elevated the intra-caecal pH in LD and LB but not in R birds ($P = 0.015$). Independent of host genotype, infection increased intra-caecal pH from 2 to 5 weeks p.i. (Fig. 6C). Moderate negative correlations between the *A. galli* larval burden and the pH of the SI were detected at 2 weeks p.i. ($r = -0.46$; $P = 0.002$) and 7 weeks p.i. ($r = -0.43$; $P = 0.003$), whereas no significant correlation was determined at other time points ($P > 0.05$). The intra-caecal pH was positively correlated with the *H. gallinarum* larval burden from 2 to 4 weeks p.i. ($r = 0.45$ to $r = 0.29$; $P < 0.05$); however, at 6 and 8 weeks p.i. moderate-negative correlations ($r = -0.38$ and $r = -0.39$, respectively, $P < 0.05$) were detected.

3.9. SCFAs and LA

The SCFA concentration in the ileum did not differ among the genotypes ($P < 0.05$), whereas LA concentration was significantly higher in R than in LD and LB birds ($P < 0.05$; Table 6). Infection had no significant effect on the concentrations of SCFA ($P = 0.878$) and LA ($P = 0.371$) in the ileum. The concentrations of SCFA ($P = 0.306$) and LA ($P = 0.339$) in the caecum were not different among the genotypes. Lower SFCA concentrations were quan-

Table 4
Size of small intestine and caeca in relation to host genotype and mixed-nematode infections in the beginning (0 weeks p.i. (wpi)) and end of the experiment (9 wpi).

		Main effects of genotypes					Infection effect				Interaction, ≤
	Item	R	LD	LB	SE	*P* ≤	Con.	Inf.	SE	*P* ≤	Inf.*Gen.
Wpi 0 (Age: 1 week)	Small intestine						–	–	–	–	–
	Length (cm)	81.8[a]	26.8[b]	27.4[b]	4.73	0.001	–	–	–	–	–
	Weight (g)	18.5[a]	8.7[b]	6.5[b]	1.21	0.001	–	–	–	–	–
	Caecum						–	–	–	–	–
	Length (cm)	8.2[ad]	6.9[abd]	5.9[b]	0.36	0.002	–	–	–	–	–
	Weight (g)	2.8[a]	1.9[b]	1.1[c]	0.19	0.001	–	–	–	–	–
Wpi 9 (Age: 10 weeks)	Small intestine										
	Length (cm)	132.9[a]	92.1[b]	85.7[b]	2.81	0.001	101.8	105.3	2.53	0.243	0.587
	Weight (g)	68.8[a]	31.0[b]	22.4[c]	2.41	0.001	40.5	41.0	2.18	0.851	0.157
	Caecum										
	Length (cm)	20.1[a]	14.9[b]	13.6[b]	0.57	0.001	17.7	14.8	0.51	0.001	0.113
	Weight (g)	19.5[a]	12.2[b]	9.7[c]	0.80	0.001	14.4	13.2	0.72	0.160	0.216

[a,b,c]Genotypes shown with different letters differ significantly (Tukey, $P < 0.05$). [d]Genotypes tend to differ (Tukey, $P < 0.10$).
Analysis for 0 wpi included 18 observations ($n = 6$ per genotype) only from the second run. Analyses for 9 wpi are based on data across spatial and temporal replications (sample size $n = 87$).
R, Ross-308; LD, Lohmann Dual; LB, Lohmann Brown Plus; Con, uninfected controls; Inf., infected; Gen, genotype.

Table 5
Morphometric measurements of the small intestine in relation to host genotype and mixed nematode infections.

	Main effects of genotypes					Infection effect				$P \leq$	Interaction, $P \leq$			
Item	R	LD	LB	SE	$P \leq$	Con.	Inf.	SE	$P \leq$	wpi	Inf.*Gen.	Inf.*wpi	Gen.*wpi	Inf.*Gen.*wpi
Villus height (μm)	1311[a]	1173[b]	1128[b]	22.0	0.001	1226	1182	18.0	0.098	0.001	0.048	0.786	0.002	0.013
Villus width (μm)	146.5[a]	144.2[a]	123.3[b]	4.10	0.001	134.7	141.3	3.34	0.172	0.001	0.440	0.341	0.041	0.335
Crypt depth (μm)	116.3	119.6	116.0	2.99	0.653	114.4	120.2	2.44	0.099	0.125	0.832	0.516	0.357	0.223
Villus:crypt ratio	11.40[a]	9.93[b]	9.82[b]	0.36	0.005	10.80	9.96	0.29	0.048	0.009	0.211	0.663	0.080	0.087

[ab]Genotypes shown with different letters differ significantly (Tukey, $P < 0.05$).
Tissue samples from jejunum were collected from six birds (three infected, three uninfected controls) of each genotype at each sampling point (week p.i.). Sample size $n = 54$ (six birds × three genotypes × three time points).
Villus:crypt ratio, villus height to crypt depth ratio; R, Ross-308; LD, Lohmann Dual; LB, Lohmann Brown Plus; Con, uninfected controls; Inf., infected; Gen, Genotype; wpi, weeks p.i.

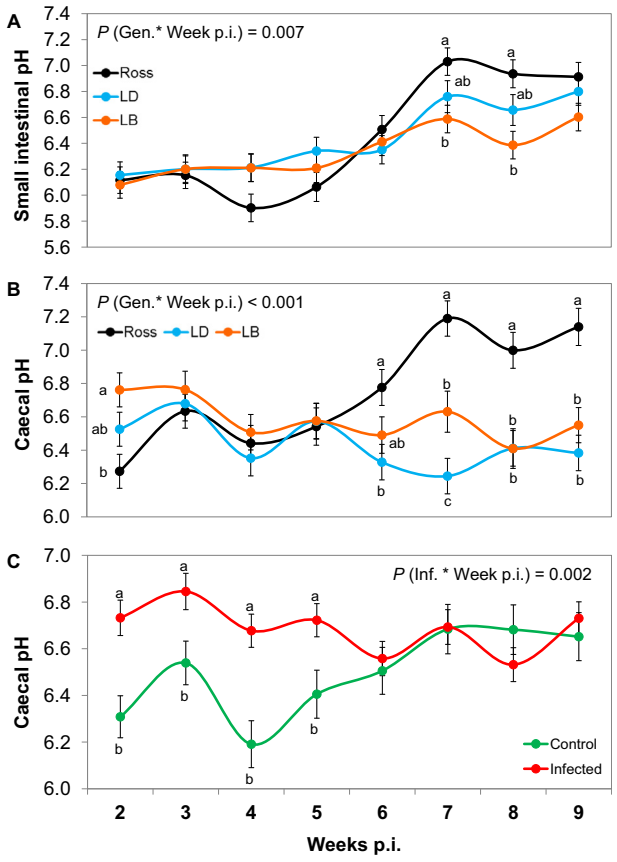

Fig. 6. Time-dependent alterations in the luminal pH of the small intestine (A) and caecum (B) in relation to chicken genotype or infection (C) effects over time. Values with different letters at the same time point indicate significant (Tukey, $P < 0.05$) differences. Sample size for each intestinal segment $n = 559$. LB, Lohmann Brown Plus; LD, Lohmann Dual; Ross, Ross-308.

tified in the infected birds than in the controls at 9 week p.i. (data not shown). Detailed results for individual SCFA and LA levels are summarized in Supplementary Table S1.

4. Discussion

To test the hypothesis that chicken genotypes selected for increased growth rate are more vulnerable to the most common nematode infections, we compared male birds of a fast growing genotype (i.e., R) with those of an extremely slow growing genotype (i.e., LB), representing layer type chickens. In addition, male birds of a dual purpose genotype (LD) with an intermediate growth potential were included in the study design. Due to sex dependency in egg production, male birds of layer type chickens are not under production pressure for what their genotype has been selected for. This applies partly to the LD birds, too. Thus, the comparison of three genotypes with extremely different growth rates provided a unique experimental model for assessing the consequences of selection for increased growth rate on response to nematode infections in chickens. The contrasted genotypes were reared in an environment designed fully for broilers (e.g. diets and husbandry conditions). Following experimental infection, measurements on host responses related to resistance, tolerance and susceptibility to mixed nematode infections were then carried out. To imitate naturally occurring field infections we used an experimental multiple species infection model with *A. galli* and *H. gallinarum*. *Heterakis gallinarum* is the main vector for transmission of *Histomonas meleagridis* (McDougald, 2005) which induces caecal lesions of similar severity in broiler and layer type chickens (Lotfi et al., 2014). We observed macroscopic caecal tissue alterations in some birds (overall <20%) that are characteristic for the protozoan parasite (McDougald, 2005; Hess et al., 2006), confirming the involvement of *H. meleagridis* in the mixed infection as it occurs under natural conditions following *H. gallinarum* infection (Grafl et al., 2011). The duration of the infection period (9 weeks)

Table 6
Biochemical parameters describing intestinal environment in ileum and caeca in divergent chicken genotypes exposed to mixed nematode infections.

	Main effects of genotypes					Infection effect					*P* values for further effects			
Item	R	LD	LB+	SE	$P \leq$	Con.	Inf.	SE	$P \leq$	wpi	Inf.*Gen.	Inf.*wpi	Gen.*wpi	Inf.*Gen.*wpi
Ileum														
SCFA[e]	2.59	2.28	2.53	0.20	0.504	2.45	2.49	0.16	0.878	0.029	0.143	0.628	0.092	0.807
Lactate[e]	40.92[a]	23.62[b]	18.36[b]	4.21	0.001	25.48	29.80	3.42	0.371	0.007	0.827	0.470	0.395	0.243
pH	6.45[ad]	6.43[a]	6.34[ad]	0.04	0.064	6.53	6.28	0.04	0.001	0.001	0.627	0.234	0.007	0.368
Caecum														
SCFA[e]	57.04	50.40	50.09	3.79	0.306	51.49	53.53	2.72	0.622	0.415	0.051	0.037	0.056	0.393
Lactate[e]	9.27	9.70	7.86	1.00	0.339	8.74	9.14	0.78	0.679	0.008	0.757	0.115	0.337	0.197
pH	6.75[a]	6.44[c]	6.59[b]	0.04	0.001	6.50	6.69	0.04	0.001	0.021	0.015	0.002	0.001	0.275

[abc]Genotypes shown with different letters differ significantly (Tukey, $P < 0.05$). [d]Genotypes tend to differ (Tukey, $P < 0.10$).
[e]Measurement unit: μmol/g digesta.
Digesta samples for short chain fatty acid concentration (SCFA) and lactate were taken from three birds per genotype and infection status at the necropsy time-points of 3, 5 and 9 weeks p.i. (wpi) ($n = 108$). Analyses on the intestinal pH are based on 559 observations.
SI: small intestine; R, Ross-308; LD, Lohmann Dual; LB+, Lohmann Brown; Con, uninfected controls; Inf., infection; Gen, genotype.

allowed one of the nematode species (*H. gallinarum*) to induce re-infection, additionally enabling an evaluation of host susceptibility to naturally occurring secondary infection.

Our results showed that tolerance to mixed nematode infections, which is the ability of the host animal to perform well despite an infection (Best et al., 2008; Råberg et al., 2009; Doeschl-Wilson and Kyriazakis, 2012), was higher in LB and LD than in R as the latter responded to infection with an impaired growth performance. This suggests that selection for increased growth rate may be associated with an impaired ability to tolerate nematode infections. Impaired tolerance in R birds was however associated with a relative nutrient scarcity due to the infection-induced lower feed intake. Resistance, the ability of the host animal to reduce the pathogen load, e.g., worm burden, FEC (Best et al., 2008; Råberg et al., 2009; Doeschl-Wilson and Kyriazakis, 2012) was dependent on both host genotype and worm species. The R was less resistant to *A. galli* compared with LB, whereas LD was in an intermediate position. These differences suggest that resistance to *A. galli* may be negatively associated with selection for increased growth rate. Resistance to *H. gallinarum* depended not only on host genotype, but also on the type of infection (i.e., primary versus secondary). *Heterakis gallinarum* burden with the first generation worms resulting from the experimental infection was similar in three genotypes. Susceptibility to naturally occurring re-infection with *H. gallinarum* was, however, higher in LB and LD than in R. In the following sections, we will address potential factors influencing tolerance, resistance and susceptibility to re-infection in different genotypes with respect to selection objectives and partitioning of resources in a given environment.

Infections reduced feed intake in all three genotypes. Mechanisms for the helminth-induced depression in feed intake are not yet conclusively elucidated. Defence strategies, actively managed by the host itself, to cope with parasites (Exton, 1997; Kyriazakis et al., 1998), changes in the hormonal regulation of feed intake (Yang et al., 1990; Zaralis et al, 2008) as well as anorexic effects stimulated by the immune system during parasite challenge (Colditz, 2008) play important roles for the impairment in the voluntary feed intake. The activation of the adaptive immune system (Colditz, 2008) and the increase in abundance of specific cytokines (e.g., IL 1, IL 6, IL 8, TNFα, IFNα) during the acute phase response are well known to induce anorexic effects (McCarthy, 2000; Plata-Salamán, 2001; Rauw, 2012). Such cytokines were also partly found to be upregulated during an *A. galli* infection in chickens (Dalgaard et al., 2015). In our study, we found the first noticeable drop in both feed intake and weight gain between 2 and 3 weeks p.i., the time period that corresponds well to the activation of the adaptive arm of the avian immune system following nematode infections (Schwarz et al., 2011a,b; Stehr et al., 2018).

Although all three genotypes responded to the infections with a reduced feed intake, only growth performance of infected R birds was impaired. In contrast to R, infected LB and LD birds were able to maintain their growth performance compared with their uninfected counterparts. The infection-induced impairment in growth performance of the fast but not that of the slower growing genotypes is in agreement with previous data from a murine-nematode system. Coltherd et al. (2009, 2011) experimentally infected mouse lines divergently selected for high or low body weight with the intestinal nematode *Heligmosomoides bakeri.* The nematode infection reduced body weight gain in the high body weight line only. Furthermore, infected mice of the high body weight line were able to maintain their growth performance when fed high but not low protein diets (Coltherd et al., 2009, 2011). Taken together, these results not only support the hypothesis that selection for high growth performance impairs host tolerance to gastrointestinal nematodes, but also provide evidence that there is a nutritional basis of tolerance to nematode infections which depends on host genotype. The finding that the infection-induced impairment in feed intake was associated with lower growth performance in the fast growing genotype but not in slower growing genotypes might indicate such an interaction. Nutrient and energy requirements of layer and broiler genotypes in the growing phase differ, with the latter requiring more nutrient-dense diets (Jeroch et al., 2013). The birds of all three genotypes were fed the same age-specific diets, which were designed in accordance with the nutrient requirements of commercial broiler chickens. This implies that excessive amounts of surplus nutrients and energy were provided to slower growing genotypes compared with the fast growing genotype. As a consequence, the nutrient and energy dense diets might have compensated the adverse effect of lower feed intake on performance of the slower growing genotypes. Surplus nutrient supply, particularly protein, indeed has great potential to compensate adverse effects of parasite infection on growth and improve resistance in different host-parasite systems (Coop and Kyriazakis, 2001; Liu et al., 2005; Coltherd et al., 2009, 2011). In line with this view, Daş et al. (2010) reported that a lysine-enriched diet reduced the impact of an *A. galli* infection on growth performance in chickens. These results collectively suggest that nutrient concentrations in excess of the physiological requirements can reduce the impact of an infection on performance traits, thereby enhancing host tolerance to parasite infection.

Feed conversion efficiency was not affected by the infections in any of the genotypes, indicating that overall nutrient utilisation was not impaired by the infection. The size of the SI, the primary absorption site of nutrients (Denbow, 2015) which is also the predilection site of *A. galli,* was not influenced by the infection. Nevertheless, structural alterations of the intestinal wall (e.g., shorter villus, mucosal thickness, mucosal lesions) during *A. galli* infection are well known (Dänicke et al., 2009; Marcos-Atxutegi et al., 2009; Luna-Olivares et al, 2015) and are likely caused by the larval stages penetrating the tissue wall (Luna-Olivares et al., 2012). In our study, infected birds tended to have a shorter villus and greater crypt depth in the jejunal section which resulted in a smaller villus:crypt ratio, but alterations were rather insubstantial as the FCR remained un-affected in all three genotypes. The higher mortality and the impaired tolerance to mixed nematode infections in R birds confirm that the fast-growing genotypes have lower fitness than slower growing genotypes (Rauw et al., 1998; Julian, 2005; Bessei, 2006; Olkowski, 2007; Raynal-Ljutovac et al., 2007; Oltenacu and Broom, 2010). This observation is in line with the theory of resource partitioning in genotypes intensively selected for a particular performance trait, i.e. increased growth rate in chickens (Rauw, 2012).

The *A. galli* burden was higher in R than in LB. Similarly, a higher number of *A. galli* survived beyond larval stages in R than in LB. The overall *A. galli* length was also higher in R than in LB birds. When compared with R and LB, LD was in an intermediate position in terms of both survival and growth of *A. galli*. These results collectively suggest that resistance to *A. galli* may be negatively associated with selection for increased growth rate in chickens. In contrast, first generation burden with *H. gallinarum*, originating from the experimental infection, was similar in the three genotypes. The larger *A. galli* burden in the fast growing R birds than in the slow growing LB birds might at least partly be due to differences in immune functions. The level of ascarid-specific IgY is not strongly associated with a direct protection in chickens (Daş et al., 2018), but is indicative of the activation of the adaptive cell-mediated immune responses (Harris and Gause, 2011; Stehr et al., 2018). Cell-mediated immune responses differ between layers and broilers following challenge with lipopolysaccharide, trinitrophenyl-conjugated keyhole limpet hemocyanin or human serum albumin (Leshchinsky and Klasing, 2001; Koenen et al., 2002; Parmentier et al., 2010). Although we did not measure

cell-mediated immune responses, differences in both infection-specific and -unspecific humoral immune responses between R and LB birds are in line with the lower resistance of R birds to *A. galli*. The LB birds tended to have higher ascarid-specific IgY levels than R birds. Similarly, the concentration of worm-unspecific IgY was higher in LB than in R, which might be associated with the lower *A. galli* burden in the slow growing genotype. The larger *A. galli* burden in R than in LB might furthermore be related to the differences in the intestinal environment of fast and slow growing genotypes. *Ascaridia galli* embeds itself in intestinal tissue (Luna-Olivares et al., 2012), likely to escape the expulsion reaction. The tissue-associated phase of *A. galli* was almost completed at approximately 5 weeks p.i. in all three genotypes. This finding is in line with previous data describing the duration of the histotrophic phase for this nematode (Herd and McNaught, 1975). A higher percentage of larvae was isolated from the tissue wall of R birds than from those of LD and LB birds. Non-larval *A. galli* counts were also higher in R than in LD and LB, likely indicating a more favourable intestinal environment for worm survival in R birds. The larger intestines as well as higher nutrient intake in R birds might have resulted in less competition among worms for space and nutrients. This hypothesis is further supported by the higher OWL of *A. galli* in R than in LB. Furthermore, the greater quantity of lactic acid in the small intestines of R birds might have been associated with the higher *A. galli* worm burdens in this genotype. The higher lactic acid concentration suggests a higher abundance of lactic acid-producing bacteria (e.g., *Lactobacillus* spp.) in the digesta, which might have promoted worm development as known for a helminth-microbiota system in mice (Reynolds et al., 2014).

Host genotype-dependent differences in resistance to *A. galli* did not apply to *H. gallinarum*, as the burden with first generation worms was similar in the three genotypes. The intra-caecal environment is known to affect larval establishment, growth and fecundity of *H. gallinarum* (Springer et al., 1970; Daş et al., 2011). Chicken genotypes differ in their intestinal environments, particularly with progressing age (Zhao et al., 2013; Schokker et al., 2015; Walugembe et al., 2015). Differences in the intra-caecal pH of the three genotypes confirmed the age-dependent differences by 6 weeks p.i. However, in the early infection period (2 weeks p.i.), there was only a transient pH difference between LB and R. The similar metabolite profiles (i.e., SCFA and LA) in the caecal digesta of different genotypes suggest negligible differences in the predilection site of *H. gallinarum*. Thus, the absence of differences in the caecal environment of the host genotypes may be associated with similar first generation burdens with *H. gallinarum* in the three genotypes.

Although the three genotypes did not differ in the number of *H. gallinarum* larvae originating from experimental infection, there were considerable differences in terms of secondary *H. gallinarum* infection (i.e. re-infection). The finding that re-infection occurred only for *H. gallinarum* but not for *A. galli* can be ascribed to the longer prepatent period of *A. galli* than that of *H. gallinarum* (Ramadan and Abou Znada, 1991; Daş et al., 2014; Daş and Gauly, 2014). The worm burden of the genotypes with first and second generation *H. gallinarum* worms provided crucial information about susceptibility to experimentally induced and naturally occurring (re)infection. When a fully controlled experimental infection was induced, the number of worms did not differ among the genotypes, whereas susceptibility to naturally occurring infection was highest in LB and lowest in R. Although secondary infections are expected to be influenced by the immune response acquired during the primary infection (Anthony et al., 2007), outcomes of naturally occurring re-infection may not be solely immune-function dependent. Unlike experimentally induced infections, the occurrence and magnitude of re-infections depend on the ingestion of embryonated eggs from the environment (i.e., from pen litter) by the host animal. The similar EPD levels in the three genotypes indicated comparable pen contamination levels with nematode eggs. This implied a similar exposure risk for the occurrence of re-infection in the three genotypes. The extremely low re-infection observed in R might have been at least partly related to the number of ingested eggs actively picked up from the pen environment, likely due to behavioural differences. In comparison to layer type chickens, broilers spend less time eating (Masic et al., 1974) and are less active (Lindqvist et al., 2006; Tickle et al., 2018). Furthermore, broilers show less ground foraging and contrafreeloading (e.g. selectively pick up from the ground) behaviours than layers (Lindqvist et al., 2006). Such behavioural differences might have influenced the number of infectious eggs taken up from the pen environment by the three genotypes, and thus may explain different levels of secondary infection with *H. gallinarum*. The finding that host responses to experimental and naturally occurring infections differed in association with host genotype is an important outcome with practical implications. Furthermore, the divergent responses of the genotypes to experimentally induced or naturally occurring infection indicate that experimentally induced infection may not necessarily be representative of naturally occurring infection. This is particularly important when host genotypes with different body sizes and behavioural patterns are compared. Thus, we propose that the assessment of host genotypes in terms of parasite resistance should not be limited to experimental infections only, but also include naturally occurring reinfection. Other factors related to experimental designs when comparing genotypes of different body size are the environmental conditions and the infection dose. We compared fast and slower growing genotypes in an environment (i.e., diet, climatic conditions, space requirements etc.) fully designed for the fast growing genotype. Despite the large differences in body size, the three genotypes were also given the same infection dose. Therefore, we appreciate that the outcomes of the experiment may differ when genotypes are constrained in genotype-specific environments (e.g. fed on genotype-specific diets) or given an infection dose adjusted to body size (e.g. see Coltherd et al., 2009, 2011). As the growth performance was only impaired in the large size broiler birds, an adjusted infection dose would even imply a higher infection pressure on R birds. However, whether growth performance of the slower growing genotypes would also be impaired by the infections, if less a nutrient- and energy-dense diet was offered, needs to be clarified in further studies.

Our data collectively suggest that resistance and tolerance to mixed nematode infections are sensitive to growth rate in chickens. These differences amongst genotypes may be partly associated with a mismatch between the actual nutrient supply and genotype-specific nutrient requirements.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2019.03.001.

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