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Research paper

Does selection for growth rate in broilers affect their resistance and tolerance to *Eimeria maxima*?

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ABSTRACT

Chickens exhibit varied responses to infection with *Eimeria* parasites. We hypothesise that broilers selected for increased growth rate will show lower resistance and tolerance to a coccidian challenge. 288 chickens of fast (F) or slow (S) growing lines were inoculated with 0 (control), 2500 (low-dose), or 7000 (high-dose) sporulated *E. maxima* oocysts at 13 days of age in two consecutive rounds. Gain and Intake were measured daily and their values relative to BW at the point of infection were calculated over the pre-patent (days 1–4 post-infection), acute (d5–8 pi), and recovery (d9–12 pi) phases of infection to assess the impact of infection. Levels of plasma carotenoids, vitamins E and A, long bone mineralisation, caecal microbiota diversity indices, and histological measurements were assessed at the acute (d6 pi) and recovery stage (d13 pi). In addition, we measured the levels of nitric oxide metabolites and the number of parasite genome copies in the jejunumat d6pi. In absolute terms F birds grew 1.42 times faster than S birds when not infected. Infection significantly reduced relative daily gain and intake ($P < 0.001$), with the effects being most pronounced during the acute phase ($P < 0.001$). Levels of all metabolites were significantly decreased, apart from NO which increased ($P < 0.001$) in response to infection on d6pi, and were accompanied by changes in histomorphometric features and the presence of *E. maxima* genome copies in infected birds, which persisted to d13pi. Furthermore, infection reduced tibia and femur mineralisation, which also persisted to d13pi. Reductions in measured variables were mostly independent of dose size, as was the level of parasite replication. The impact of infection was similar for S and F-line birds for all measured parameters, and there were no significant interactions between line x dose size on any of these parameters. In conclusion, our results suggest that line differences in productive performance do not influence host responses to coccidiosis when offered nutrient adequate diets.

1. Introduction

Genetic selection for production traits, to meet increased requirements for chicken meat, has been applied to broiler chickens at an unprecedented rate (Siegel, 2014; Tixier-Boichard et al., 2012; Zuidhof et al., 2014). Such an emphasis on productive traits may have compromised the ability of modern broilers to cope with metabolic and skeletal disorders (Dawkins and Layton, 2012; Julian, 1998) and infectious pathogens (Cheema et al., 2003; Yunis et al., 2000). This raises concerns amongst the general public and have led, for example, the Dutch Organisation of Retailers to take the strategic decision that they will only sell chicken meat from slow-growing animals. Similar trends appear in other parts of the European Union (van der Aar et al., 2016).

The hypothesis is that when resources are limited, as is in the case of most health challenges, birds from lines selected for productivity will continue to direct these resources to productive rather than functional traits, such as the ability to cope with disease (Coop and Kyriazakis, 1999). This is a consequence of the genetic drive for greater productivity (Rauw, 2012). Here, we used two modern broiler lines that have been selected for different growth rates to test the hypothesis that selection for growth will penalise bird resistance to parasite infection to a greater extend (Coop and Kyriazakis, 1999). A lower level of resistance could potentially affect markers of tolerance, such as the magnitude and duration of pathogen induced anorexia, in such a way

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that less resistant hosts could show a delayed induction of anorexia, which is of longer duration and of smaller magnitude (Doeschl-Wilson et al., 2009; Lough et al., 2015). Host resistance has been defined as the mechanism by which the entry and/or the replication of pathogens within the host is restricted, with tolerance defined as the host's ability to limit the detrimental effect of pathogens on performance without necessarily affecting pathogen burden (Doeschl-Wilson and Kyriazakis, 2012; Lough et al., 2015; Rauw, 2012). Although in broilers monospecific coccidian infections rarely occur in the field, a controlled coccidial infection is a good model, to test our hypothesis as the main effects are a reduction in food intake (Kipper et al., 2013; Preston-Mafham and Sykes, 1970) and absorption of nutrients (Persia et al., 2006; Preston-Mafham and Sykes, 1970; Su et al., 2014), leading to reduced availability of nutrient resources. We used infection with *Eimeria maxima* to test our hypothesis, one of the most commonly encountered coccidia spp. The magnitude of its effects depends on the degree of tissue damage and inflammation (Lillehoj and Trout, 1996; Williams, 2005), typically occuring around the period of maximum parasite schizogony and gametogony (Hein, 1968), which coincides with shortening of the villi and enlargement of crypts.

To assess tolerance we measured performance over the course of infection. In addition, we implemented two sampling points, one at the acute ((d6 post-infection (pi)) and one at the recovery stage of infection (d13pi) to measure plasma levels of lutein and zeaxanthin, which are the major carotenoids in cereal grains (Humphries and Khachik, 2003) and fat-soluble vitamins retinol (vitamin A) and α-tocopherol (vitamin E). Reduced plasma levels of both carotenoids and fat-soluble vitamins may serve as indicators of intestinal epithelial damage and may be used as markers of severity for coccidial infections (Allen et al., 2004; Allen and Fetterer, 2002a; Singh and Donovan, 1973). Furthermore, histological measurements were carried to directly assess the level of damage induced to the intestinal mucosa. Fast and slow growing broilers may differ on the level of long bone mineralisation (Williams et al., 2004) and coccidiosis has been shown to affect aspects of bone development (Fetterer et al., 2013). To that end we also assessed long bone mineralisation at both d6 and d13pi. Plasma levels of nitric oxide (NO) metabolites were also assessed at the acute stage of infection as they constitute a marker of the severity of coccidial infections (Allen, 1997a,b). They facilitate parasite killing (Lillehoj and Li, 2004), but their excessive production contributes to the pathology of *E. maxima* (Allen and Fetterer, 2002b) infections due to oxidative damage and their concentration is negatively correlated with average daily gain (ADG) and carotenoid concentration at d6pi (Zhu et al., 2000). Even though *E. maxima* does not replicate in the caeca it was hypothesised that infection in the small intestine might impact the caecal microbiota due to reduced nutrient absorption resulting in increased nutrients in the caeca, whilst differences between genetic lines of chicken have been previously observed (Schokker et al., 2015). In assessing the differences in resistance of our treatment groups, we estimated the number of parasite genome copies in the jejunum, the primary site of *E. maxima* colonisation and replication, at the peak of parasite replication (*i.e.* d6pi; (Blake et al., 2006)) and by proxy accounting for all possible underlying immune responses.

2. Materials and methods

2.1. Chicken management

All procedures were conducted under the UK Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63/EU for animal experiments and carried out under Home Office authorization (P441ADF04). The experiment was conducted over two rounds, separated by 6 weeks. Each round consisted of 72 male day-old chicks of a fast-growing line (Ross 308, F), and an equal number of a slow growing line (Ross Ranger Classic, S). All birds were obtained from the same hatchery and had parents subjected to the same husbandry regime. Furthermore, the same parent stock flocks were used for each of the two lines which aged 37 and 43 weeks of age for round A and B, respectively. The growth potential of these lines differs by approximately 25%, according to the performance objectives of the breeding company. Lines F and S originate from the same paternal lines but different maternal lines; growth rate is not part of the selection criteria for the maternal lines of the S line.

Birds were housed in a windowless, thermostatically controlled room in 24 circular pens with a diameter of 1.2 m (1.13 m^2). Pens were equipped with tube feeders and bell-drinkers, and wood shavings were used as litter to a depth of 5 cm. Birds had *ad libitum* access to feed and water throughout the trial. The temperature within the pen was monitored daily and maintained to meet recommendations for spot brooding (Aviagen, 2014b), starting at 34 °C at chick placement and was gradually reduced to 20 °C by 25 days of age. Light intensity at pen level ranged from 180 to 220 lux, while a lighting schedule of 23L:1D was applied for the first 7 days of age and switched to 18L:6D for the remainder of the trial.

Starter (d0–10) and grower (d11–26) diets were manufactured according to Aviagen nutrition specifications (Aviagen, 2014a) and were offered to both lines (Table 1). The starter diet was offered in crumb form and the grower in pelleted form.

2.2. Experimental design and inoculations

This experiment followed a 3 × 2 factorial design with coccidian infection and bird line as the independent variables, while the experimental round was treated as a blocking factor. Upon arrival, day-old chicks of each line were randomly assigned to one of three treatment groups. Each group consisted of 8 replicate pens, and initial stocking density was 6 birds per pen. Birds were orally inoculated at 13 days of age (experimental day 0) with a single 0.5 ml dose of H_2O (control group, C), 2500 (low-dose group, L) or 7000 (high-dose group, H) sporulated *E. maxima* oocysts of the Weybridge laboratory reference strain. Bird weight was measured at placement and bird weight and

Table 1
Ingredient and calculated chemical composition of the starter (d0–10) and grower (d11–26 post-hatch) diets.

Item	Starter	Grower
Ingredient (%)		
Wheat	47.8	51.5
Soybean meal (48 % CP)	32	25.2
Corn	10	10
Soybean full fat	4.0	7.0
Dicalcium phosphate	1.89	1.66
Soy crude oil	1.84	2.32
Limestone	0.64	0.59
Vitamin and mineral premix[1]	0.4	0.4
DL methionine	0.33	0.30
L-Lysine	0.27	0.24
Sodium bicarbonate (27 %)	0.21	0.19
Sodium chloride (39 %)	0.19	0.20
L-Threonine	0.14	0.12
Choline chloride (60 %)	0.05	0.05
L-Valine	0.03	0.02
Xylanase[3]	0.02	0.02
Calculated nutrient composition (%)		
ME (kcal/kg)	3,000	3,100
Crude protein	23.5	21.7
Crude fat	4.37	5.41
Calcium	0.96	0.87
Phophorus	0.76	0.70
Available phosphorus	0.48	0.44
Ash	5.23	4.78

[1,2]Provided per kilogram of feed vitamins, minerals and digestible AA according to Aviagen Nutrient specifications (Aviagen, 2014a).
[3]Ronozyme® WX, DSM Nutritional Products Ltd.