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Effects of rearing environment on the gut antimicrobial responses of two broiler chicken lines

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ABSTRACT

To reduce the risk of enteric disease in poultry, knowledge of how bird gut innate defences mature with age while also responding to different rearing environments is necessary. In this study the gut innate responses of two phylogenetically distinct lines of poultry raised from hatch to 35 days, in conditions mimicing high hygiene (HH) and low hygiene (LH) rearing environments, were compared. Analyses focussed on the proximal gut antimicrobial activities and the duodenal and caecal AvBD1, 4 and 10 defensin profiles.

Variability in microbial killing was observed between individual birds in each of the two lines at all ages, but samples from day 0 birds (hatch) of both lines exhibited marked killing properties, Line X: 19 ± 11% (SEM) and Line Y: 8.5 ± 12% (SEM). By day 7 a relaxation in killing was observed with bacterial survival increased from 3 (Line Y (LY)) to 11 (Line X (LX)) fold in birds reared in the HH environment. A less marked response was observed in the LH environment and delayed until day 14. At day 35 the gut antimicrobial properties of the two lines were comparable.

The AvBD 1, 4 and 10 data relating to the duodenal and caecal tissues of day 0, 7 and 35 birds LX and LY birds revealed gene expression trends specific to each line and to the different rearing environments although the data were confounded by inter-individual variability. In summary elevated AvBD1 duodenal expression was detected in day 0 and day 7 LX, but not LY birds, maintained in LH environments; Line X and Y duodenal AvBD4 profiles were detected in day 7 birds reared in both environments although duodenal AvBD10 expression was less sensitive to bird age and rearing background. Caecal AvBD1 expression was particularly evident in newly hatched birds.

These data suggest that proximal gut antimicrobial activity is related to the bird rearing environments although the roles of the AvBDs in such activities require further investigation.

1. Introduction

Newly hatched and young birds rely on their innate defences for protection against infection and with the onset of food intake the gut is particularly susceptible to microbial assault. The inability or failure of the gut defences to protect against pathogens can present as chronic gut inflammation (Ramasundara et al., 2009), which in commercial rearing environments can escalate into disease and bird mortality. Hence an understanding of the innate immune responses of the bird gut to microbial challenges, particularly those relating to the immediate rearing environment, is necessary to help direct future breeding programmes.

Microbial colonisation of the chicken gut is naturally associated with inflammation linked potentially to the induction of pro-inflammatory cytokines that help prime the gut immune system and facilitate the maturation of the gut (Crhanova et al., 2011). During this period immediate gut protection is mediated through the collective functioning of the gut innate defences including the epithelial barrier, the mucus layer covering the epithelium and the production of proteins and peptides with antimicrobial activity. These host molecules including lysozyme, sPLA2, and the defensins function as endogenous antibiotics with the avian defensins (AvBDs) providing a significant protective barrier due to their broad-spectrum antimicrobial activity against bacteria and fungi, a function linked to their structure and charge (Cuperus et al.,

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Table 1
End-point PCR (SQ) and QPCR (Q) primers. AvBD1-10 primers utilised for semi-quantitative (SQ) and quantitative analyses (Q), optimal annealing conditions and product size.

Gene	Forward primer	Reverse primer	Annealing temperature (°C)	Length (bp)
AvBD1SQ/Q (NM_204993.1)	TACCTCTGCTGCAAAAGAATATGG	GAGAAGCCAGGGTGATGTCC	60	70
AvBD2SQ (NM_204992)	TGCTGCAAATGGCCTTGGAAT	CTTCTTGCTGCTGAGGCTTTG	63	113
AvBD3SQ (NM_204650.2)	CTGCTGTGGAAGAGCATATGAGGT	CTTCCACTGCCACGGTCATAC	61	142
AvBD4SQ/Q (NM_001001610.2)	TGCTGTAGATGGTTGTAGTGTGAA	ACCGGTACAATGGTTCCCCA	61	100
AvBD5SQ (NM_001001608.2)	GCAAGAAAGGAACCTGCCCT	GCAAGAAAGGAACCTGCCCT	64	136
AvBD6SQ (NM_001001193)	TCTTGCTGTGTGAGGAACAGG	TTAGAGTGCCAGAGAGGCCA	61	95
AvBD7SQ (NM_001001194.1)	CTCTTGCTGTGCAAGGGGAT	GGAGTGCCAGAGAAGCCATT	59	91
AvBD8SQ (NM_001001781.1)	TGCCGGACTGTGTACGACTAA	TTCAGCCCCAAATTCCAGGTT	58	112
AvBD9SQ (NM_001001611.2)	GCAAAGGCTATTCCACAGCAGA	CTTCTTGGCTGTAAGCTGGAGCA	62	103
AvBD10SQ/Q (NM_001001609.1)	CTGTTAAACTGCTGTGCCAAGATTC	TGTTGCTGGTACAAGGGCAAT	58	77

2013). Potential immunomodulatory functions also support a role for the AvBDs in the recruitment of immune cells thus facilitating the development of the adaptive immune response (Soman et al., 2009).

In birds, as well as mammals, microbial colonisation of the avian gut occurs in conjunction with defensin gene expression (Bar-Shira and Friedman, 2006; Crhanova et al., 2011; Salzman and Bevins, 2013), which supports a role for the encoded peptides in controlling the indigenous microbial numbers and composition. Following acute microbial challenges the gut defensin responses appear less predictable with studies in birds reporting both up and down regulation of the genes (Akbari et al., 2008; Hong et al., 2016; Meade et al., 2009a; Milona et al., 2007). In reality a variety of factors in addition to the microbial challenge combine to affect gene expression including the location of the tissues along the anterior-posterior axis of the bird gut as well as the age and breed of the birds studied. In fact the importance of bird genetics in the hierarchical functioning of the gut innate defences is illustrated by the identification of AvBD single-nucleotide polymorphisms (SNPs) as molecular markers for selecting poultry resistant to enteric pathogens including *Salmonella enteritidis* (Hasenstein and Lamont, 2007).

The genetic selection of poultry is often compromised by the rearing environment yet to preserve the elite stocks, environmental challenges that influence bird performance and direct genetic selection are often performed in carefully monitored biosecure conditions that support a high hygiene (HH) environment. For some genetic traits this scenario works well with the selection against foot-pad dermatitis in a HH environment also reducing FPD prevalence in birds reared in commercial or low hygiene (LH) conditions (Kapell et al., 2012). However, in relation to genes associated with growth and/or immunity the outcomes appear less transferable. Examination of 12 immune related genes with performance and mortality traits in elite commercial broiler lines revealed that the *TGF-β3-MSp1* SNP was significantly associated with mortality in HH, but not LH environments, while progeny of birds with allele 1 of *iNOS-Alu1* had a higher 40 day body weight in HH compared to LH conditions (Ye et al., 2006). These data show that a better understanding of interactions between genetic and environmental factors is necessary to underpin the selection of bird stocks with immune systems more robust to environmental changes.

The immune responsiveness of young birds is important in selecting and maintaining healthy birds yet the involvement and roles of the host innate defences, particularly the defensins, in protecting birds against disease in low hygiene (LH) environments more reflective of conditions in commercial situations are limited. To address this, two phylogenetically distinct genetic lines of poultry were raised from hatch to 35 days, in controlled conditions mimicing HH and LH environments and the effects of rearing environment on the gut innate responses of such birds compared. The primary focus was the upper gastrointestinal antimicrobial activities and the duodenal and caecal defensin profiles.

2. Materials and methods

2.1. Birds

Two phylogenetically distinct lines of poultry used in broiler breeding and designated X and Y were studied (Andreescu et al., 2007). The birds, 100 in total and 50 per line, were housed on farms in two different environments: a high biosecure environment referred to as pedigree or high hygiene (HH) where breeding programme candidates are selected and a non bio-secure environment referred to as sib-test or low hygiene (LH) environment resembling commercial conditions. Water and high-quality diet were provided ad libitum throughout the growing period. Litter was in the form of wood shavings. Following hatch male birds were randomly selected and reared for up to 35 days under conditions of HH or LH. In the LH locations birds were raised in barns containing a mix of old (mechanically conditioned) and new litter while in the HH barns a complete disinfection process was adopted between stocking regimes and all pens were supplied with fresh litter. Tissue sampling was performed at day 0 (hatch), 7, 14 and 35 respectively. On the day of bird sampling a representative bedding sample of shavings combined with faecal matter (20 g) was taken from each pen. Samples were analysed by Poultry Health Service Ltd, UK for colony forming bacteria (CFU) per gram of sample.

2.2. Gut antimicrobial assays

At each sampling time the duodenal loop of each bird was excised, cut longitudinally, the gut contents removed by washing in 0.1 M phosphate buffered saline (PBS) and the mucosal layer collected by scraping. Samples were frozen in liquid nitrogen and stored at −80 °C. Cationic gut proteins were extracted in 10% acetic acid and following lyophilisation each sample was reconstituted in 0.1 M PBS pH 7.4. Antimicrobial assays were performed as described previously (Townes et al., 2004), using *Salmonella enterica* serovar Typhimurium *pho*P (Behlau and Miller, 1993), and data normalised to protein concentration. Gut antimicrobial activity was presented as percentage of killed bacteria compared to PBS controls.

2.3. LC–MS/MS analysis

Four volumes of ice-cold acetone were added to each reconstituted gut sample, the samples gently vortexed and stored overnight at −20 °C. Following centrifugation the supernatants were removed, and the reconstituted residues subjected to LC/MS analysis (NEPAF Proteome Facility – now Newcastle University Protein & Proteome Analysis (NUPPA)). Annotation of the proteins was achieved through searching and mapping of the LC/MS