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Original Research Article

An early feeding regime and a high-density amino acid diet on growth performance of broilers under subclinical necrotic enteritis challenge

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ABSTRACT

Broilers that have early access to feed have been shown to have enhanced immune system and gut development and heightened resilience against necrotic enteritis (NE). This study examined the effect of early feeding a high amino acid density diet on performance of broilers under a sub-clinical NE challenge model. Ross 308 broilers ($n = 576$) were assigned to a $2 \times 2 \times 2$ factorial design with 2 feeding regimes (feed access either within 6 h post-hatch or after 48 h post-hatch), 2 diets (control diet or the control diet with an additional 10% digestible amino acids [HAA]) and either presence or absence of NE challenge. Oral administrations of *Eimeria* species (d 9) and a field strain of *Clostridium perfringens* (d 14) were used to induce NE. Broiler performance was analysed at d 13, 23, 30 and 35. Intestinal lesion score and bacterial count were analysed on d 16. The NE challenge reduced overall bird performance and induced severe intestinal lesions, without causing notable mortality. At d 23 bird weight was significantly lower ($P < 0.001$) in the challenged birds compared with the unchallenged birds, but by d 30 the challenged birds had recovered and challenge no longer had an impact on bird performance. Birds fed the HAA diet had greater body weight by d 35 and heightened *Lactobacillus* content in the ileum at d 16 ($P < 0.05$). Birds that were fed the HAA diet after a period of fasting performed better in terms of feed conversion ratio (FCR) under challenge. The findings from this study suggest there are beneficial effects of feeding high amino acid diets to birds in response to external stresses, such as post-hatch fasting and subclinical NE.

1. Introduction

Necrotic enteritis (NE) is a multifactorial, bacterial borne enteric disease that causes devastating losses to poultry flocks (up to 30% mortality in an infected flock), costing approximately US$2 billion per annum worldwide (Dahiya, 2006; Van Immerseel et al., 2009; Wade and Keyburn, 2015). The causative agent is *Clostridium perfringens*; a Gram-positive, anaerobic, rod spore-forming bacterium that is ubiquitous and is capable of producing a variety of extracellular toxins and invasive enzymes (Gibert et al., 1997; Keyburn et al., 2010). Its pathogenic state can be triggered from predisposing factors that disturb the integrity of the gut mucosa; such as increased viscosity of intestinal digesta, high level of protein in the diet and coccidiosis infection (Collier et al., 2008; Rodgers et al., 2014). The clinical form of NE results in significant necrosis of the small intestine and hence catastrophic levels of mortality (Kaldhusdal and Løvland, 2000; Van Immerseel et al., 2009). The subclinical form exhibits less obvious signs, with initial symptoms of just slightly reduced growth due to impaired nutrient utilisation from damaged intestinal mucosa. As a result there is often delayed onset of effective treatment and spreading amongst the flock, resulting in a substantial loss to production (Skinner et al., 2010; Van der Sluis, 2000).

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C. perfringens strains have been shown to be susceptible to various anti-microbial drugs in both *in vitro* and *in vivo* studies (Devriese et al., 1993; Geier et al., 2010). However, worldwide concerns about the emergence of antimicrobial resistant bacteria strains mean that alternatives to in-feed antibiotics in animal production, that can both combat bacterial infections and replace antibiotic growth promoters, are desperately being sought after (Bedford, 2000; Choct, 2001; Dibner and Richards, 2005; Ferket, 2004; Huyghebaert, 2011). Replacements for antibiotics that can reduce the prevalence of *C. perfringens* induced NE must therefore be established.

Bacterial pathogens in the gut thrive in the presence of imbalanced feed compositions. For example, a diet with high crude protein or soluble non-starch polysaccharides causes increased gastrointestinal transit time, which induces mass proliferation of opportunistic *C. perfringens* in the microflora (Annett et al., 2002; Choct and Annison, 1992; Moore, 2016; Riddell and Kong, 1992; Tech, 1999; Wu et al., 2014). A refined nutritional formula that makes the broiler gut environment less susceptible to *C. perfringens* domination is therefore critical for maintaining optimal health and performance in broiler chickens and reducing production costs (Croom et al., 2000; Dahiya, 2006; Moore, 2016; Van Immerseel et al., 2009).

The amount of time between hatch and access to feed has been shown to greatly influence survivability and performance in young birds (Geyra et al., 2001). Due to the nature of the broiler industry, birds often do not get access to water and feed for up to 48 h post-hatch (Dibner et al., 1998). Early nutrient availability, especially access to essential amino acids, could play a significant role in immune development (Ao et al., 2002; Kidd, 2004; Li et al., 2007). Ao et al. (2002) reported that birds that had early access to feed and water post-hatch had improved immune development and better performance in the presence of NE than birds that had been fasted for 48 h post-hatch. Essential amino acids have been shown to be important components in development of the immune system in birds and amino acid utilisation is prioritised towards tissues involved in immune response and inflammation (Li et al., 2007; Le Floc'h et al., 2004). Kidd et al. (2004) reported that healthy broilers responded positively to high dietary amino acid inclusion, and the positive effect on performance was more evident in birds exposed to the dietary treatment immediately post-hatch. The impact of amino acid supplementation in young broilers is still however under debate and the relationship between amino acids and NE is poorly understood. The aim of this study was to examine the benefit of early access to feed and of feeding starter diets with a high amino acid density on the response of birds to NE challenge.

2. Materials and methods

2.1. Experimental design and feeding treatments

A total of 576 day-old male broiler chickens (Ross 308) were procured from Baiada Country Road Hatchery (Tamworth, NSW, Australia) at the day of hatch. Chicks were randomised by weight and placed in floor pens (approx. area 120 cm × 75 cm per pen), bedded on clean wood shavings. Birds were vaccinated against Marek's disease, infectious bronchitis, and Newcastle disease at the hatchery before arrival. The lighting regimen used was 23, 18 and 23 h of light during days 0 to 24, 25 to 30 and 31 to 35, respectively. The floor bedding temperature was maintained at 34 to 35 °C from d 0 to 3 and was then gradually decreased by 3 °C per week onward until 22 to 24 °C was reached by d 21. The rearing facility at the University of New England meets the Australian standards, and the area was sterilised before bird arrival. All experimental procedures involved in this study were approved by the Animal Ethics Committee of the University of New England. The study had a 2 × 2 × 2 factorial design, resulting in 8 treatments; 2 feeding regimes of either immediate access to feed (FED) or access to feed delayed by 48 h post-hatch (HELD), 2 starter diets with (control) or without amino acid density 10% above the recommended level (HAA) and either exposed to NE challenge or not (Challenged or Unchallenged). Birds were allocated to 48 pens with 12 birds per pen and 6 replicates per treatment (72 birds/treatment). Birds were evenly distributed to ensure that there were no statistical differences between initial starting pen weights.

Birds in the FED treatment group had *ad libitum* access to feed on arrival (within 6 h post-hatch), whereas the birds in the HELD treatment group had access to only water on arrival and feed was then introduced after 48 h post-hatch. The starter diets were fed as crumble until d 7 and then as pellet (⌀2 to 3 mm). The diets were wheat, sorghum, meat meal and soybean based and were formulated based on Aviagen (2012) nutritional specification guidelines. The HAA starter diet was the same as the control diet but with an additional 10% digestible amino acids (Table 1). From d 13 all birds were fed the same grower diet until d 24 and then the same finisher diet from d 24 to 35, both fed as pellets (⌀3 to 3.5 mm).

2.2. Necrotic enteritis challenge

On day 9, each bird in the NE-challenge group was given 1 mL per os vaccine strain of *Eimeria* (Bioproperties Pty Ltd., Sydney, Australia). Each 1 mL gavage included phosphate buffered saline (PBS) suspension of approximately 5,000 oocysts each of *Eimeria acervulina* and *Eimeria maxima*, and 2,500 oocysts of *Eimeria brunetti*. To the unchallenged group, 1 mL of sterile PBS was

Table 1
Starter diet formulation and nutrient composition.

Item	Control	HAA
Ingredient, %		
Wheat	30	30
Sorghum	26.6	21
Soy bean meal	30.1	36.1
Canola meal solvent extracted	3	1
Meat and bone meal	2	3.8
Canola oil	4.3	4.7
Limestone	1.2	1
Dical Phos 18P/21Ca	1,476	1,089
NaCl	0.128	0.101
Na bicarb	0.2	0.2
UNE vitamin premix[1]	0.05	0.05
UNE trace mineral premix[2]	0.075	0.075
Choline Cl 70%	0.038	0.045
Analysed composition, %		
ME, kcal/kg	3,025	3,027
Crude protein	22.94	25.34
Crude fat	6.21	6.65
Crude fibre	3.00	3.03
Digestible amino acids		
Isoleucine	1.01	1.11
Arginine	1.34	1.52
L-lysine	1.27	1.40
DL-methionine	0.60	0.67
Methionine + cystine	0.94	1.03
Tryptophan	0.23	0.26
L-threonine	0.83	0.91
Valine	0.94	1.03

HAA = high amino acid starter diet (10% more essential amino acid over the Aviagen recommendations); UNE = University of New England.

[1] Vitamin premix per kg contains: vitamin A, 12 MIU; vitamin D, 5 MIU; vitamin E, 75 mg; vitamin K, 3 mg; nicotinic acid, 55 mg; pantothenic acid, 13 mg; folic acid, 2 mg; riboflavin, 8 mg; cyanocobalamin, 0.016 mg; biotin, 0.25 mg; pyridoxine, 5 mg; thiamine, 3 mg; antioxidant, 50 mg.

[2] Mineral premix per kg contains: Cu, 16 mg as copper sulphate; Mn, 60.

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