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Original Research Article

Effects of yeast cell wall on growth performance, immune responses and intestinal short chain fatty acid concentrations of broiler in an experimental necrotic enteritis model

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ABSTRACT

Subclinical necrotic enteritis (NE) causes devastating economic losses in the broiler chicken industry, especially in birds raised free of in-feed antibiotics. Prebiotics are potential alternatives to in-feed antibiotics. Yeast cell wall extract (YCW) derived from *Saccharomyces cerevisiae* is a prebiotic with known immune modulating effects. This study examined the effects of YCW and antibiotics (AB) during subclinical NE on broiler growth performance, intestinal lesions, humoral immune response and gut microflora metabolites. The study employed a 2×3 factorial arrangement of treatments. Factors were: NE challenge (yes or no) and feed additive (control, AB, or YCW). Each treatment was replicated in 8 floor pens with 15 birds per pen. Challenged birds had higher feed conversion ratio (FCR) than unchallenged birds on d 35 ($P < 0.05$). Dietary inclusion of AB decreased FCR regardless of challenge ($P < 0.05$) on d 24 and 35. Inclusion of YCW reduced serum interleukin-1 (IL-1) concentration in NE challenged birds ($P < 0.01$) and increased immunoglobulin (Ig) G ($P < 0.05$) and Ig M ($P < 0.05$) levels compared to other dietary treatments regardless of challenge. Yeast cell wall extract increased formic acid concentration in cecal contents during challenge and increased butyric acid concentration in unchallenged birds on d 16. This study indicates YCW suppressed inflammatory response, promoted generation of immunoglobulin and increased short chain fatty acid production suggesting potential benefits to bird health.

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1. Introduction

Necrotic enteritis (NE) caused by *Clostridium perfringens* (Cp) is an economically important disease of the broiler industry. In-feed antibiotics have largely controlled NE. The ban of in-feed antibiotics in the European Union has resulted in an increased incidence of NE (Shojadoost et al., 2012). This disease is estimated to cost the global poultry industry approximately 6 billion US dollars annually (Wade and Keyburn, 2015) with the subclinical form of this disease more devastating than the acute form (Timbermont et al., 2011). Subclinical NE persists in flocks without detectable clinical signs and results in a drag on performance, i.e., lower weight gain and increased feed conversion ratio (FCR), due to poor digestion and absorption of nutrients (Van der Sluis, 2000; Kaldhusdal et al., 2001). There is current heightened interest in replacements for in-feed antibiotics to control subclinical NE.

Yeast cell walls (YCW) contain mannoproteins, β (1,3)-glucans, β (1,6)-glucans, chitin and glycophospholipid surface proteins associated with the plasma membrane. The YCW is known to have prebiotic properties with efficacy for modulating immunity and gut microflora (Pourabedin and Zhao, 2015). As such, YCW is a potential replacement for dietary sub-therapeutic antibiotic. Immunomodulation has been suggested to be the key mode of action of YCW (Shashidhara and Devegowda, 2003; Gao et al., 2008). Prebiotics derived from YCW have been found to promote the production of immunoglobulin (Ig) (Czech et al., 2010) that plays as an important part in host immunity. By recognizing and binding particular antigens, Ig controls the prevalence and severity of infections

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(Goudswaard et al., 1977; Desmidt et al., 1998). Prebiotics derived from YCW were also found to prevent the disease by inducing the pro-inflammatory responses (Monsan and Paul, 2008) as inflammation mediates the host immunity to against the disease such as acute bacterial infection (Medzhitov, 2007). The mode of action of YCW also involves altering gut microflora composition via competitive exclusion (Callaway et al., 2008), production of antimicrobial agents (Chen et al., 2007; Muñoz et al., 2012) and changing the fermentation pattern of the gut microflora (Donalson et al., 2008).

A previous study showed improved performance of broilers under clinical NE with dietary addition of YCW (M'Sadeq et al., 2015). However, the effect of YCW during subclinical NE has not been investigated to the best of our knowledge. Therefore, the present study was designed to examine the role of YCW in performance, intestinal NE lesions, pro- and anti-inflammatory markers, Ig production and intestinal metabolite profile in the broiler chickens challenged by subclinical NE.

2. Materials and methods

This study was conducted at University of New England, Armidale, New South Wales, Australia. All experimental procedures and protocols involved in this study were reviewed and approved by the Animal Ethics Committee of the University of New England. Birds were cared for according to the Australian Code for the Care and Use of Animals for Scientific Purposes (8th Edition, 2013).

2.1. Experimental design, diet and bird husbandry

The Animal Ethics Committee of University of New England (Australia) reviewed and approved all experimental procedures involved in this study. Ross 308 male broiler chicks ($n = 720$) were obtained from a commercial hatchery (Baiada Hatchery, Tamworth, NSW, Australia). Birds were vaccinated against Marek's disease, infectious bronchitis and Newcastle disease at the hatchery. Chicks were weighed, allocated to floor pens (120 cm × 75 cm per pen) such that starting pen weights were not different ($P > 0.05$). Fresh wood shavings were used as bedding. The environmentally controlled room was disinfected and preheated prior to chick arrival. The lighting and temperature program followed the breeder recommendations (Aviagen, 2014).

The study employed a 2 × 3 factorial arrangement of treatments with 8 replicate pens per treatment and 15 birds per pen. Factors were: NE challenge (no or yes), and additive (none [control], YCW [Actigen, Alltech, USA; 800, 400, 200 g/t; starter, grower and finisher, respectively], or antibiotics [AB]). The AB treatment consisted of a combination of Zn bacitracin and salinomycin. Dosage was Albac G 150 (Zoetis Australia Pty Ltd, Rhodes, NSW) at 100 g/t starter, 50 g/t grower and finisher; Sacox 120 Microgranulate (Huvepharma, Burwood, VIC) at 60 g/t. The diets were based on wheat, soybean meal, sorghum, canola meal, meat and bone meal and formulated to meet Ross 308 nutrient specifications (Table 1). The starter diet was fed from d 0 to 10, grower from d 10 to 24 and finisher from d 24 to 35. The starter diet was crumbled to d 7 and thereafter pellets were fed (2.5 mm). Pellet temperature was 65 °C. Birds had *ad libitum* access to feed and water. Pen weight and cumulative pen feed intake were recorded on d 0, 24 and 35 and used to calculate mean bird weight gain (BWG), feed intake (FI) and FCR (corrected for mortality).

2.2. Necrotic enteritis challenge

On d 9, each bird in the NE-challenge group was given 1 mL *per os* vaccine strains of *Eimeria* (Bioproperties Pty Ltd., Sydney, Australia). Each 1 mL gavage included phosphate buffered saline (PBS) suspension of approximately 5,000 oocysts each of *Escherichia acervulina* and *Eimeria maxima*, and 2,500 oocysts of *Escherichia brunetti*. To the unchallenged group, 1 mL of sterile PBS was administered as control. On d 14 and 15, each bird in the NE-challenge group was given 1 mL of Cp suspension at a concentration of 10^8 cfu/mL. A primary poultry isolate of Cp type A strain EHE-NE36 (CSIRO Livestock Industries, Geelong, Australia) was incubated overnight at 39 °C in 100 mL of sterile thioglycollate broth (USP alternative, Oxoid, Australia) followed by subsequent overnight incubations of 1 mL of the previous culture in 100 mL of sterilized cooked meat medium (Oxoid, Australia), and then in 500 mL of thioglycollate broth containing starch (10 g/L) and pancreatic digest of casein (5 g/L) to obtain the challenge inoculum. Birds in the unchallenged groups received 1 mL of sterile thioglycollate broth. Unchallenged and challenged birds were physically partitioned to prevent cross contamination.

Table 1
Ingredient and nutrient composition of experimental basal diets (as-fed, % unless otherwise noted).

Item	Starter	Grower	Finisher
Ingredients			
Sorghum	20.0	20.0	20.0
Wheat	38.5	43.6	50.6
Soybean meal	26.2	18.1	10.5
Canola meal	5.0	7.0	7.0
Meat and bone meal	3.0	4.0	6.0
Canola oil	3.79	4.79	4.44
Limestone	0.83	0.63	0.32
Dicalcium phosphate[1]	1.05	0.60	–
Allzyme SSF	0.20	0.20	0.20
NaCl	0.17	0.14	0.11
Na bicarbonate	0.20	0.15	0.15
Vitamin mineral premix[2]	0.20	0.20	0.20
Choline Cl 60%	0.74	0.73	0.65
L-lysine	0.361	0.317	0.277
DL-methionine	0.358	0.289	0.214
L-threonine	0.199	0.165	0.128
Calculated composition			
ME, kcal/kg	3,025	3,150	3,200
Crude protein	22.5	20.6	19.3
DLys	1.27	1.10	0.94
DMet + DCys	0.94	0.84	0.73
DThr	0.83	0.073	0.63
DIle	0.85	0.77	0.67
DArg	1.31	1.14	0.99
DVal	0.98	0.84	0.79
Crude fat	6.05	7.18	7.05
Crude fiber	2.63	2.65	2.59
Calcium	0.90	0.80	0.70
Available phosphorus	0.45	0.40	0.36
Total phosphorus	0.72	0.67	0.62
Sodium	0.18	0.16	0.16
Chloride	0.25	0.22	0.20
Choline	1,600	1,500	1,400
Linoleic acid	1.86	2.11	2.01

[1] Dicalcium phosphate contained: phosphorus, 18%; calcium, 21%.
[2] Vitamin and mineral concentrate supplied per kilogram diet: retinol, 12,000 IU; cholecalciferol, 5,000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 μg; biotin, 200 μg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulfate), 16 mg; Fe (sulfate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulfate and oxide), 120 mg; Zn (sulfate and oxide), 100 mg.

2.3. Sampling and lesion scoring

On d 16, 2 birds per pen were randomly selected and euthanized by cervical dislocation. The liver was excised and weighed. The size of the liver was expressed relative to the whole bird body weight. Blood samples were collected in non-heparinized tubes by

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