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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 broilers 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.



## 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 |
| $CaHPO_4$[1] | 1.05 | 0.60 | – |
| Allzyme SSF | 0.20 | 0.20 | 0.20 |
| NaCl | 0.17 | 0.14 | 0.11 |
| $NaHCO_3$ | 0.20 | 0.15 | 0.15 |
| Vitamin mineral premix[2] | 0.20 | 0.20 | 0.20 |
| Choline chloride 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 |
| Dlle | 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

puncturing the brachial vein and centrifuged at 2,000 × *g* for 10 min to obtain serum. The ceca content and serum were pooled from 2 birds in each replicate pen and stored at −20 °C until further analysis. The entire length of the section of small intestine (duodenum, jejunum and ileum) of all sampled birds underwent a lesion scoring process, based on a previously reported lesion scoring system that ranges from 0 to 4 (Prescott et al., 1978; Broussard et al., 1986). Three experienced personnel, with no knowledge of the trial design, were involved in the scoring process.

### 2.4. Serum interleukins and immunoglobulins ELISA assays

Commercially available ELISA kits were used to measure the serum interleukin-1 and 10 (IL-1, IL-10) and serum IgA, IgG and IgM in serum collected at d 16 following the manufacturer instructions (IL-1, IL-10 ELISA: Cusabio, Wuhan, China, catalog No. CSB-E10069Ch; CSB-E12835C; IgA, IgG and IgM ELISA: Abnova, Taipei, Taiwan, catalog No. KA2426; KA2031; KA2427).

### 2.5. Short chain fatty acids (SCFA)

Gas chromatography was used to analyze SCFA in the caecal content following the modified version of the method described by Jensen et al. (1995). One milliliter of internal standard (0.01 mol/L ethylbutyric acid) was added to approximately 2 g of fresh homogenized digesta sample and the solution was then mixed and centrifuged at 27,216 × *g* at 5 °C for 20 min. Approximately 1 mL of the resulting supernatant, 0.5 mL of concentrated HCl, and 2.5 mL of ether were then combined. An internal standard solution and a blank were also prepared using 1 mL of the standard acid mixture and 1 mL of water respectively in place of the supernatant. The mixture was then centrifuged at 2,000 × *g* at 5 °C for 15 min and 400 μL of the resulting supernatant was combined with 40 μL of N-tert-butyldimethlsilyl-N-methyltrifluoroacetamide. The samples were then heated at 80 °C for 20 min, left at room temperature for 48 h and were then analyzed on a Varian CP3400 CX gas Chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA). Short chain fatty acids concentrations were presented as μmol/g digesta.

### 2.6. Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics package version 22 (IBM Corporation). The study employed a 2 × 3 factorial arrangement of treatments in a completely randomized design. Factors were NE challenge (no or yes), and additives (none, YCW and AB). Main effects and interactions were examined by analysis of variance, using the General Linear Model. Mortality and intestinal lesion score data were analyzed by the nonparametric Kruskal–Wallis test, as the data were not normally distributed. Treatment means were separated using Tukey HSD *post hoc* test where appropriate. Statistical significance was declared at $P < 0.05$.

## 3. Results

### 3.1. Performance

Table 2 shows BWG, FI, FCR and livability results from d 0 to 24. Challenge of NE negatively affected performance from d 0 to 24. Body weight gain of the challenged birds was 93% of that of the unchallenged birds (1,267 vs. 1,356 g, $P < 0.001$). Challenge had no impact on FI, but FCR was 6 points higher in challenged birds compared to controls ($P < 0.001$). There was a challenge × additive interaction for weight gain ($P < 0.05$) from d 0 to 24. Feeding AB improved BWG in challenged birds and were not different to unchallenged birds. Feed conversion ratio was also lower ($P < 0.01$) in the birds fed AB diets compared to those fed the control diet or diet with YCW. Treatment had no significant effect on livability from d 0 to 24.

Cumulative performance from d 0 to 35 is presented in Table 3. The long-term effect of NE was observed, as reflected by the higher FCR (3 points, $P < 0.05$) in the challenged compared to the unchallenged birds. Across challenge groups, FCR was 5 points lower in birds fed the diet supplemented with AB compared to those fed the control diet or diet with YCW ($P < 0.01$). Treatment had no effect on weight gain or FI from d 0 to 35. No challenge × additive interactions were observed ($P > 0.05$).

### 3.2. Intestinal lesion scores

Intestinal lesion scores in the duodenum, jejunum and ileum measured at d 16 are presented in Table 4. Birds in all treatments had low lesions scores indicating the challenge was subclinical. However, a challenge × additive interaction ($P < 0.01$) was detected, indicating higher ileal lesion scores in the challenged birds fed the control diet compared to the challenged birds fed the AB diet. Challenged birds fed YCW did not have different lesion scores from AB fed birds or control birds.

### 3.3. Humoral immune response and relative liver weights

Responses of cytokines (IL-1 and IL-10), Ig (IgA, IgG and IgM) in the peripheral blood and relative liver weights on d 16 are presented in Table 5. A challenge × additive interaction ($P < 0.05$) was observed for serum IL-1 levels. Serum IL-1 concentration was lower in the challenged birds fed YCW as compared to control or AB but no effect of additive treatment was observed in unchallenged birds. Necrotic enteritis challenge led to higher serum IL-1 concentration in both control and AB fed birds but not in those fed YCW. Neither challenge nor additive had a significant effect on IL-10 concentration.

Elevated levels of IgG were detected in the NE challenge group compared to unchallenged group ($P < 0.01$). Regardless of challenge, birds supplemented with YCW had higher IgG ($P < 0.05$) and IgM ($P < 0.05$) levels compared to groups fed the control diet or diet supplemented with AB. Treatment had no significant effect on serum IgA level ($P > 0.05$).

A challenge × additive interaction ($P < 0.001$) was observed for liver weight. In challenged birds, liver weights of AB fed birds were higher than bird fed YCW or controls whereas in unchallenged birds liver weights were not different ($P > 0.05$) among additives.

### 3.4. Short chain fatty acids

Table 6 shows the concentrations of 9 SCFA measured in the ceca at d 16. Challenge × additive interactions were observed for formic acid ($P < 0.05$) and butyric acid ($P < 0.05$). Without challenge, both AB and YCW fed groups showed higher cecal formic acid concentrations compared to controls, and under NE challenge, the birds fed the AB diet showed lower cecal formic acid concentrations than YCW fed birds but no difference from birds fed the control diet. Challenged birds had lower levels of formic acid compared to unchallenged birds across additive groups. In unchallenged birds, cecal butyric acid levels of YCW fed birds were higher than birds fed AB or controls but there were no additive effects in challenged birds. Cecal propionic acid concentrations were higher ($P < 0.01$) and valeric acid concentrations lower ($P < 0.01$) in unchallenged birds compared to challenged birds. Isovaleric acid concentration was higher ($P < 0.05$) in birds fed the control diet or YCW compared to AB.

**Table 2**
Performance of broilers from d 0 to 24.

| Interactions | | Feed intake, g/bird | Weight gain, g/bird | FCR, g/g | Livability, % |
|---|---|---|---|---|---|
| Challenge | Additives[1] | | | | |
| No | None | 1,710 | 1,351[a] | 1.265 | 98 |
| No | YCW | 1,698 | 1,356[a] | 1.251 | 99 |
| No | AB | 1,692 | 1,361[a] | 1.243 | 98 |
| Yes | None | 1,653 | 1,241[b] | 1.333 | 98 |
| Yes | YCW | 1,643 | 1,242[b] | 1.324 | 95 |
| Yes | AB | 1,684 | 1,320[a] | 1.279 | 97 |
| SEM | | 10.9 | 9.7 | 0.006 | 0.6 |
| Main effects | | | | | |
| Challenge | | | | | |
| No | | 1,700 | 1,356[a] | 1.253[b] | 98 |
| Yes | | 1,660 | 1,267[b] | 1.312[a] | 97 |
| Additives | | | | | |
| None | | 1,682 | 1,296[b] | 1.299[a] | 98 |
| YCW | | 1,671 | 1,299[b] | 1.288[a] | 97 |
| AB | | 1,688 | 1,341[a] | 1.261[b] | 98 |
| *P*-value | | | | | |
| Challenge | | 0.072 | <0.001 | <0.001 | 0.168 |
| Additive | | 0.803 | 0.012 | 0.002 | 0.791 |
| Challenge × Additive | | 0.590 | 0.046 | 0.170 | 0.369 |

FCR = feed conversion ratio; YCW = yeast cell wall extract; AB = antibiotics.
[a, b] Means not sharing the same superscripts are significantly different ($P < 0.05$).
[1] None = basal diet (no additive); YCW = basal diet + YCW (Actigen, Alltech, USA); AB = basal diet + Zn bacitracin (Albac 150) + salinomycin (Sacox 120).

**Table 3**
Performance of broilers from d 0 to 35.

| Interactions | | Feed intake, g/bird | Weight gain, g/bird | FCR, g/g | Livability, % |
|---|---|---|---|---|---|
| Challenge | Additives[1] | | | | |
| No | None | 3,601 | 2,501 | 1.441 | 98 |
| No | YCW | 3,600 | 2,516 | 1.431 | 97 |
| No | AB | 3,505 | 2,521 | 1.390 | 98 |
| Yes | None | 3,668 | 2,501 | 1.466 | 98 |
| Yes | YCW | 3,680 | 2,499 | 1.474 | 95 |
| Yes | AB | 3,591 | 2,539 | 1.414 | 95 |
| SEM | | 23.2 | 14.5 | 0.006 | 0.6 |
| Main effects | | | | | |
| Challenge | | | | | |
| No | | 3,568 | 2,513 | 1.421[b] | 98 |
| Yes | | 3,646 | 2,513 | 1.451[a] | 96 |
| Additives | | | | | |
| None | | 3,634 | 2,501 | 1.454[a] | 98 |
| YCW | | 3,640 | 2,508 | 1.453[a] | 96 |
| AB | | 3,548 | 2,530 | 1.402[b] | 97 |
| *P*-value | | | | | |
| Challenge | | 0.098 | 0.995 | 0.004 | 0.186 |
| Additive | | 0.199 | 0.723 | <0.001 | 0.399 |
| Challenge × Additive | | 0.986 | 0.894 | 0.689 | 0.607 |

FCR = feed conversion ratio; YCW = yeast cell wall extract; AB = antibiotics.
[a, b] Means not sharing the same superscripts are significantly different ($P < 0.05$).
[1] None = basal diet (no additive) YCW = basal diet + YCW (Actigen, Alltech, USA); AB = basal diet + Zn bacitracin (Albac 150) + salinomycin (Sacox 120).

## 4. Discussion

In this study, only AB supplementation improved performance of broilers under subclinical NE challenge. A successful subclinical NE was induced as confirmed by poor performance, presence of mild lesions and lack of mortality (Gholamiandehkordi et al., 2007). In a previous study, both YCW and AB improved performance (FI, BWG, FCR) during clinical NE challenge (M'Sadeq et al., 2015). This might suggest that the intensity of NE challenge affects the efficacy of YCW. Other evidence exists showing YCW had little effect on performance loss during Cp or coccidial infection (Hofacre et al., 2003; Nollet et al., 2007; Ao et al., 2012; Shanmugasundaram et al., 2013). This low impact of YCW on intestinal lesion scores in this study was similar to that reported by Ao et al. (2012) and Hofacre et al. (2003). However M'Sadeq et al. (2015) observed improved performance by YCW under clinical NE challenge, it did not influence intestinal lesion scores. It is logical that alleviation of intestinal lesions would relieve the impact of NE because intact gut morphology would maintain nutrient absorption. Perhaps YCW does not affect the colonization of Cp on the intestinal epithelium directly, i.e., lesion development, but rather may have other roles, for example, immunity of the birds, that determine different performance responses in clinical or subclinical NE outbreaks.

Immunomodulation as a result of dietary YCW has been postulated and the current study showed YCW exhibited anti-inflammatory effects and promoted Ig production. Inflammation is an important but often overlooked consequence of NE. This study showed that NE elevated the pro-inflammatory cytokine level, which is supported by previous reports (Park et al., 2008). Inflammation can reset priority of physiological and developmental processes to prepare the host to fight infection (Klasing, 1988). Specifically, inflammation impairs FI, absorption of nutrients

**Table 4**
Duodenum, jejunum and ileum NE lesion score at d 16.

| Interactions | | Duodenum | Jejunum | Ileum |
|---|---|---|---|---|
| Challenge | Additives[1] | | | |
| No | None | 0.00 | 0.00 | 0.00[b] |
| No | YCW | 0.00 | 0.00 | 0.00[b] |
| No | AB | 0.00 | 0.00 | 0.00[b] |
| Yes | None | 0.00 | 0.13 | 0.47[a] |
| Yes | YCW | 0.09 | 0.13 | 0.25[ab] |
| Yes | AB | 0.03 | 0.00 | 0.13[b] |
| SEM | | 0.016 | 0.025 | 0.047 |
| Main effects | | | | |
| Challenge | | | | |
| No | | 0.00 | 0.00 | 0.00[b] |
| Yes | | 0.04 | 0.08 | 0.28[a] |
| Additives | | | | |
| None | | 0.00 | 0.06 | 0.23 |
| YCW | | 0.05 | 0.06 | 0.13 |
| AB | | 0.02 | 0.00 | 0.06 |
| *P*-value | | | | |
| Challenge | | 0.153 | 0.077 | <0.001 |
| Additive | | 0.600 | 0.367 | 0.228 |
| Challenge × Additive | | 0.537 | 0.211 | 0.003 |

YCW = yeast cell wall extract; AB = antibiotics.
[a, b] Means not sharing the same superscripts are significantly different ($P < 0.05$).
[1] None = basal diet (no additive); YCW = basal diet + YCW (Actigen, Alltech, USA); AB = basal diet + Zn bacitracin (Albac 150) + salinomycin (Sacox 120).

**Table 5**
Serum interleukins response (IL-1 and IL-10, pg/mL), immunoglobulins (IgA, IgG and IgM, mg/mL) and average liver weights as a percent of live body weight (g/g, %) of broilers at d 16.

| Interactions | | IL-1 | IL-10 | IgA | IgG | IgM | Liver |
|---|---|---|---|---|---|---|---|
| Challenge | Additives[1] | | | | | | |
| No | None | 103[b] | 1.13 | 4.7 | 3.4 | 1.7 | 3.33[cd] |
| No | YCW | 99[b] | 1.15 | 6.1 | 4.1 | 2.1 | 3.32[cd] |
| No | AB | 102[b] | 1.17 | 4.0 | 3.3 | 1.9 | 3.28[d] |
| Yes | None | 120[a] | 1.16 | 6.3 | 3.9 | 1.8 | 3.59[b] |
| Yes | YCW | 106[b] | 1.16 | 6.1 | 4.5 | 2.3 | 3.50[bc] |
| Yes | AB | 126[a] | 1.23 | 5.1 | 4.3 | 1.6 | 4.03[a] |
| SEM | | 1.9 | 0.024 | 0.38 | 0.12 | 0.08 | 0.053 |
| Main effects | | | | | | | |
| Challenge | | | | | | | |
| No | | 101[b] | 1.15 | 5.0 | 3.6[b] | 1.9 | 3.31[b] |
| Yes | | 117[a] | 1.18 | 5.8 | 4.2[a] | 1.9 | 3.71[a] |
| Additives | | | | | | | |
| None | | 112[a] | 1.15 | 5.5 | 3.6[b] | 1.8[b] | 3.46[b] |
| YCW | | 103[b] | 1.15 | 6.1 | 4.3[a] | 2.2[a] | 3.41[b] |
| AB | | 114[a] | 1.20 | 4.5 | 3.8[b] | 1.7[b] | 3.66[a] |
| *P*-value | | | | | | | |
| Challenge | | <0.001 | 0.522 | 0.263 | 0.002 | 0.983 | <0.001 |
| Additive | | 0.002 | 0.698 | 0.267 | 0.018 | 0.012 | 0.004 |
| Challenge × Additive | | 0.040 | 0.921 | 0.734 | 0.469 | 0.318 | <0.001 |

YCW = yeast cell wall extract; AB = antibiotics.
[a–d] Means not sharing the same superscripts are significantly different ($P < 0.05$).
[1] None = basal diet (no additive); YCW = basal diet + YCW (Actigen, Alltech, USA); AB = basal diet + Zn bacitracin (Albac 150) + salinomycin (Sacox 120).

anabolic processes in order to divert nutrients toward tissues involved in immunity, fever and hepatic production of acute phase protein (Klasing et al., 1987; Klasing, 2005). This implies that the cost of NE consists of 2 parts – a dysfunctional intestine and the cost of immunity respectively. The cost of immunity may contribute more to productivity loss during subclinical NE when intestinal damage is mild (Gholamiandehkordi et al., 2007) and thus anti-inflammation could be expected to resolve loss from subclinical NE. It is interesting to find that YCW showed stronger anti-inflammatory effects than AB or control diet in the present study, which was also reflected in the lower liver relative weight because enlarged liver can be induced by system inflammation (Lichtman et al., 1990). Ferket et al. (2002) also observed birds fed YCW product had lighter liver than those fed a diet with antibiotics under lipopolysaccharide challenge. Yeast cell wall extract has also been reported to terminate systemic inflammation earlier than virginiamycin in the findings of Baurhoo et al. (2012). It is likely that YCW competitively excludes pathogens whose cell integrity will be maintained whereas the use of antibiotics results in bacterial fragmentation during cytolysis (Yamasaki et al., 2009). This would lead to release of cellular contents, including microbial DNA and prototypical enterotoxins (Su et al., 2006) thus inducing inflammatory response. On the other hand, in this study YCW promoted Ig secretion to be suggested that might offer protection against the infection (Cetin et al., 2005; Czech et al., 2010). Moreover, YCW increased butyric acid and formic acid production in the ceca. To some extent, SCFA control bacterial infections, act as potential antibiotic replacers, and benefit the gut health as an energy source for enterocytes (Hernández et al., 2006; García et al., 2007; Fernández-Rubio et al., 2009).

**Table 6**
Short chain fatty acids levels in caecal contents at d 16 (μmol/g digesta).

| Interactions | | Formic | Acetic | Propionic | Isobutyric | Butyric | Isovaleric | Valeric | Lactic | Succinic |
|---|---|---|---|---|---|---|---|---|---|---|
| Challenge | Additives[1] | | | | | | | | | |
| No | None | 0.73$^{b}$ | 57.81 | 21.66 | 0.86 | 11.71$^{b}$ | 0.31 | 1.13 | 0.61 | 3.86 |
| No | YCW | 1.05$^{a}$ | 76.92 | 30.08 | 1.02 | 18.73$^{a}$ | 0.38 | 1.67 | 0.83 | 1.93 |
| No | AB | 1.19$^{a}$ | 68.23 | 25.78 | 0.61 | 15.00$^{b}$ | 0.20 | 1.27 | 1.04 | 3.31 |
| Yes | None | 0.43$^{cd}$ | 82.19 | 14.66 | 0.85 | 21.50$^{a}$ | 0.31 | 2.29 | 0.67 | 2.17 |
| Yes | YCW | 0.66$^{bc}$ | 74.20 | 15.38 | 0.71 | 20.72$^{a}$ | 0.20 | 1.97 | 0.53 | 4.14 |
| Yes | AB | 0.31$^{d}$ | 83.01 | 15.29 | 0.59 | 21.05$^{a}$ | 0.16 | 2.13 | 1.18 | 3.35 |
| SEM | | 0.064 | 3.189 | 1.600 | 0.054 | 0.719 | 0.023 | 0.115 | 0.088 | 0.524 |
| Main effects | | | | | | | | | | |
| Challenge | | | | | | | | | | |
| No | | 0.99$^{a}$ | 67.66 | 25.84$^{a}$ | 0.83 | 15.15$^{b}$ | 0.30 | 1.36$^{b}$ | 0.82 | 3.03 |
| Yes | | 0.47$^{b}$ | 79.80 | 15.11$^{b}$ | 0.72 | 21.09$^{a}$ | 0.22 | 2.13$^{a}$ | 0.79 | 3.22 |
| Additives | | | | | | | | | | |
| None | | 0.58$^{b}$ | 70.00 | 18.16 | 0.85 | 16.60$^{b}$ | 0.31$^{a}$ | 1.71 | 0.64 | 3.02 |
| YCW | | 0.86$^{a}$ | 75.56 | 22.73 | 0.87 | 19.73$^{a}$ | 0.29$^{a}$ | 1.82 | 0.68 | 3.04 |
| AB | | 0.75$^{ab}$ | 75.62 | 20.53 | 0.60 | 18.03$^{ab}$ | 0.18$^{b}$ | 1.7 | 1.11 | 3.33 |
| *P*-value | | | | | | | | | | |
| Challenge | | <0.001 | 0.058 | 0.005 | 0.281 | <0.001 | 0.085 | 0.005 | 0.854 | 0.863 |
| Additive | | 0.044 | 0.693 | 0.414 | 0.069 | 0.045 | 0.037 | 0.856 | 0.057 | 0.965 |
| Challenge × Additive | | 0.024 | 0.208 | 0.533 | 0.424 | 0.010 | 0.251 | 0.218 | 0.551 | 0.352 |

YCW = yeast cell wall extract; AB = antibiotics.
[a-d] Means not sharing the same superscripts are significantly different ($P < 0.05$).
[1] None = basal diet (no additive); YCW = basal diet + YCW (Actigen, Alltech, USA); AB = basal diet + Zn bacitracin (Albac 150) + salinomycin (Sacox 120).

There could be numerous reasons why YCW did not improve performance during subclinical NE. It is possible that YCW supplementation suppressed inflammation temporarily but pro-inflammatory response started to occur after continuously feeding of YCW. Prolonged provision of dietary YCW increased the IL-6 level, a pro-inflammatory cytokine, a week after Cp challenge (Ao et al., 2012). Tzianabos (2000) also suggested β-glucan derived from YCW is potent to prime and activate inflammatory responses. Thus, it may suggest that feeding YCW may help the bird adjust its priority of response, i.e., anti- or pro-inflammation, depending on the lumen environment or severity of the disease, which may largely determine the performance response (Klasing et al., 1987). This hypothesis can be supported by the findings of Huff et al. (2006) who found β-glucan only improved performance of broilers during an *Escherichia coli* challenge and such improvement diminished when β-glucan was given to the challenged birds continuously. Moreover, in the same study, β-glucan reduced BW without the presence of challenge that suggested stimulating immunity by β-glucan was costly to production and may need to be cautious to use under a challenge-free environment. This might explain why YCW is more effective on performance under clinical rather than subclinical NE challenge when the syndrome is milder and recovery is faster. Furthermore, it is logical that Ig secretion is an energetically expensive. The proliferation of Ig secreting lymphocytes was promoted under a conventional husbandry condition but not under a germ-free, antigen-free practices where results in better growth performance (Hooijkaas et al., 1984; Furuse and Yokota, 1985; Pereira et al., 1986; Bos et al., 1988; Bakker et al., 1995). Moreover, SCFA are not necessarily beneficial to gut integrity and production as Nafday et al. (2005) and Liu et al. (2014) proved colonic mucosal injury and weight loss can be mediated by administration of SCFA such as butyric acid in rats or goat. It was of interest that in the current experiment YCW increased butyrate levels in the hindgut in the unchallenged group yet resulted in increased formate in the hindgut of challenged birds. It was hypothesized that the increased butyrate caused by YCW in unchallenged birds may be metabolized into formate during challenge. Further work is warranted to test this hypothesis.

Gut health management is an important strategy to maintain productivity in the poultry industry in the post-antibiotic era. The use of prebiotics is one such strategy that has gained attention over recent years (Patterson and Burkholder, 2003) but there remains tremendous variation in their efficacy (Santin et al., 2001; Benites et al., 2008; Midilli et al., 2008). There are many possible reasons for this variability in efficacy, including the type of prebiotics used, the gut microflora, feed, chicken breed, age of birds, husbandry environment, and management practice. More work is required to elucidate each of these variables in more extensive settings.

## 5. Conclusion

Supplementation of YCW may offer protection against subclinical NE due to its effects on anti-inflammation, immunoglobulin secretion and SCFA production. However, continuous feeding of YCW, especially after birds have recovered from subclinical NE, may diminish the positive effect of YCW on performance. The current study showed the range and regime of YCW that may be deployed under antibiotic-free production situations.

## Conflict of interest

The authors declare that they have no conflict of interest.

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