Influence of prebiotic yeast cell wall extracts on growth performance, carcase attributes, biochemical metabolites, and intestinal morphology and bacteriology of broiler chickens challenged with *Salmonella typhimurium* and *Clostridium perfringens*

Manal M. Alkhulaif, Abdulmohsen H. Alqhtani, Abdulrahman S. Alharthi, Ali R. Al Sulaiman and Alaeldein M. Abudabos

ABSTRACT

The current experiment was conducted to examine the efficacy of yeast cell wall (YCW) supplementation on growth performance, carcase characteristics, serum biochemistry, ileum histological structure, and caecum bacterial populations of broilers subjected to *Salmonella typhimurium* (*S. typhimurium*) and *Clostridium perfringens* (*C. perfringens*) challenges from 11 to 35 d of age. A total of 360 mixed-sex Ross 308 broilers were randomly distributed to 5 treatments with 12 replicates of 6 birds each as follows: control with no additive or challenge; *S. typhimurium* challenge; *C. perfringens* challenge; *S. typhimurium* + YCW; *C. perfringens* + YCW. The YCW was supplemented at a level of 0.5 g/kg. Compared to the control group, the unsupplemented challenged groups had deteriorated average daily gain, feed conversion ratio (FCR), and European production efficiency factor (EPEF) during all experimental periods (*p* < .001) and reduced dressing percentage, glucose (GLU) concentration (*p* < .001), and *Lactobacillus* population (*p* < .01). Moreover, the *S. typhimurium*-challenged group had reduced total protein (TP) level and elevated *S. typhimurium* count (*p* < .001), while the *C. perfringens*-challenged group showed decreased liveability (*p* < .001) and crypt depth (*p* < .05) and increased *C. perfringens* count (*p* < .01) than the control group. Supplementation of YCW under *S. typhimurium* challenge improved FCR and EPEF during all experimental periods (*p* < .001), levels of TP, GLU (*p* < .001), and globulin (*p* < .05), villus height (VH) (*p* < .001), and villus surface area (*p* < .01) and reduced *S. typhimurium* count (*p* < .01). Broilers fed YCW and challenged with *C. perfringens* exhibited improved FCR during grower and overall periods, EPEF during all experimental periods, liveability, GLU level, and VH (*p* < .001). In conclusion, dietary supplementation of YCW could ameliorate the harmful impacts of disease challenges on the growth efficiency of broilers.

HIGHLIGHTS

- *Saccharomyces cerevisiae* yeast-derived prebiotics have been experimented and utilised as a potential alternative to antibiotics in poultry diets.
- Challenge with *Salmonella typhimurium* or *Clostridium perfringens* diminished growth efficiency and compromised intestinal health in broiler chickens.
- The dietary supplementation of yeast cell wall could reduce the negative effects of pathogens on broiler performance.

Introduction

*Salmonella* is a Gram-negative infectious bacterium that targets the avian caecum, and *Salmonella* is known to cause low growth performance, high mortality, and serious economic losses to the worldwide poultry industry (Tellez et al. 2001; Revolloedo et al. 2009). In addition, *Salmonella* is associated with foodborne outbreaks in humans due to the consumption of poultry products contaminated with *Salmonella* (Mughini-Gras et al. 2014). Among the most common enteric pathogenic *Salmonella* serotypes bacteria isolated from broiler chickens is *Salmonella* enterica serovar *Typhimurium* (*S. typhimurium*) (Finstad et al. 2012). Due to the current strict regulations on using...
antibiotics as growth promoters in the poultry industry to control bacterial resistance to antibiotics in addition to the consumers’ need for antibiotics-free poultry, and considering *S. typhimurium* have the potential to develop antimicrobial resistance more quickly than other *Salmonella* species (Sharan et al. 2011; Wang et al. 2019), a number of feed additives have been explored to reduce the *Salmonella* colonisation and maximise growth performance in broiler chickens (Gadde et al. 2017; Bilal et al. 2021).

*Clostridium perfringens* (*C. perfringens*) is a Gram-positive, rod-shaped, spore-forming, and anaerobic pathogenic bacteria that induce several avian diseases known for the damage of intestinal mucosa such as necrotic enteritis (NE) in broiler flocks between 2–6 weeks of age (Porter 1998; Cooper and Songer 2009; Moore 2016). The NE is one of the most common bacterial diseases in the poultry industry resulting in up to 30% mortality in broiler chickens (Songer 1996; Cooper and Songer 2009). The economic losses attributed to NE in the global poultry industry have been estimated to be around 6 billion US dollars per year (Xue et al. 2018), basically due to the costs of treatment and the reductions in growth performance (Lovland and Kaldhusdal 2001; Van Immerseel et al. 2004). For more than four decades, antibiotic growth promoters were supplemented to poultry feed and water to protect them from pathogenic bacteria such as *C. perfringens* (Dahiya et al. 2006). The concerns about the spread of antibiotic resistance markers (Wierup 2001; Thompson et al. 2006) have restricted the usage of antibiotics in the global poultry industry (Jesudhasan et al. 2021), leading to an increase in the prevalence of NE in poultry flocks from 4% to 12% (Van Immerseel et al. 2004; Hermans and Morgan 2007; Van Immerseel et al. 2009). Therefore, it has become necessary to find novel dietary approaches to eliminate *C. perfringens*, and eventually control NE development in broiler chickens.

In the context of moving towards antibiotic-free poultry production, several studies explored the potential effects of different feed additives on eliminating the enteric pathogenic bacteria, in addition to improving growth performance and health status in broiler chickens (Stevanović et al. 2018; Selaledi et al. 2020; Mahfuz et al. 2021). Among these feed additives, yeast cell wall (YCW) extract from *Saccharomyces cerevisiae* (*S. cerevisiae*) has been studied as a potential antibiotic alternative in challenged broiler chickens with bacterial infections (Xue et al. 2017; Bilal et al. 2021; Schwartz and Vetvicka 2021). The YCW contains mannan-oligosaccharides, β-glucans such as β (1,3)-glucans and β (1,6)-glucans, in addition to other compounds like mannan-proteins, chitin, and glycophosholipid surface proteins (Xue et al. 2017). The various YCW fractions could modulate birds’ intestinal health through different mechanisms (Pascual et al. 2020). For example, dietary supplementation with YCW improved gut integrity by decreasing the number of pathogens in the ileum and caecum (Li et al. 2017). Furthermore, YCW can partially protect the intestinal health of broilers challenged with NE by improved the populations of *Lactobacilli* and *Bifidobacteria* and reduced the colonisation of *C. perfringens* (Liu et al. 2018). Similarly, the sequencing findings by Bi et al. (2020) showed that YCW supplements modulated the gut microbiota in chickens through augmented abundance of Ruminococcaceae and diminished Bacteroidaceae populations of caecal content. In addition, feeding YCW lowered the severity of intestinal lesions of NE-challenged broilers through the enhancement of cell growth and survival signalling, along with a decrease in apoptosis (Johnson et al. 2020). Based on the aforementioned studies, we hypothesised that dietary YCW could exert beneficial effects on the health and performance of broiler chickens under bacterial challenges. Therefore, the present study was performed to evaluate the potential prebiotic effects of supplemental *S. cerevisiae* cell wall on growth performance, carcase traits, blood biomarkers, and intestinal structure and bacterial colonisation of grower and finisher broilers challenged by the pathogenic *S. typhimurium* and *C. perfringens* bacteria.

Materials and methods

The animal use protocol was approved by the Research Ethics Committee of King Saud University, Riyadh, Saudi Arabia.

Husbandry and treatments

A total of 360 1-day-old mixed-sex broiler chicks (Ross 308) with similar body weights were obtained from a commercial hatchery and randomly allocated to battery cages in an environmentally controlled poultry unit in groups of 6 chicks (3 males and 3 females) per cage at a stocking density of 30 kg/m². All chicks were confirmed for the absence of *S. typhimurium* and *C. perfringens* as previously described (Abudabos et al. 2018) and fed on a commercial starter diet (3000 kcal/kg ME and 23% CP) till 10 d of age. A standard grower (11–25 d) and finisher (26–35...
Table 1. Ingredient and nutritional composition of the experimental diets (as-fed basis).

| Ingredient (%)             | Grower (11-25 d) | Finisher (26-35 d) |
|----------------------------|------------------|--------------------|
| Yellow corn                | 57.4             | 61.0               |
| Soybean meal               | 33.0             | 32.3               |
| Wheat bran                 | 3.00             | 0.00               |
| Choline chloride 60%       | 0.05             | 0.00               |
| Corn oil                   | 3.00             | 3.35               |
| Dicalcium phosphate        | 1.77             | 1.62               |
| Ground limestone           | 0.85             | 0.80               |
| Salt                       | 0.30             | 0.40               |
| DL-methionine              | 0.29             | 0.26               |
| Lysine-HCL                 | 0.19             | 0.11               |
| Vitamin-Mineral premix*    | 0.20             | 0.20               |
| Total                      | 100              | 100                |

Calculated analysis

| Metabolizable energy, kcal/kg | 3100 | 3200 |
| Crude protein, %             | 21.5  | 20.0 |
| Non phytate P, %             | 0.44  | 0.41 |
| Calcium, %                   | 0.87  | 0.81 |
| Lysine, %                    | 1.15  | 1.06 |
| Sulfur amino acids, %        | 0.87  | 0.83 |
| Threonine, %                 | 0.77  | 0.71 |

*Vitamin-mineral premix contains the following per kg: vitamin A, 12000000 IU; vitamin D3, 5000000 IU; vitamin E, 800000 IU; vitamin K3, 3200 mg; vitamin B1, 3200 mg; vitamin B2, 8600 mg; vitamin B3, 65000 mg; vitamin B5, 20000 mg; vitamin B6, 4300 mg; vitamin B7, 220 mg; vitamin B9, 2200 mg; vitamin B12, 17 mg; copper, 16000 mg; iodine, 1250 mg; iron, 20000 mg; manganese, 120000 mg; selenium, 300 mg; zinc, 110000 mg.

d) diets with isocaloric and isonitrogenous contents were offered in mash form based on a corn-soybean meal and were formulated to meet the recommendations of Ross broilers (Aviagen 2019). The composition of the experimental diets is shown in Table 1. Birds were offered ad libitum access to feed and water and grown in accordance with standard breeding practices (Aviagen 2018) during the entire rearing period.

On d 11 of age, each cage was assigned to 1 of 5 treatment groups in a completely randomised design as follows: control group without bacterial challenge or feed additive; challenge group with oral inoculation of *S. typhimurium* (ATCC# 14028) at a rate of \(3 \times 10^9\) colony-forming units (CFU)/mL; challenge group with oral inoculation of *C. perfringens* (Microbiologics Inc., Cloud, MN, USA) at a rate of \(4 \times 10^8\) CFU/mL; *S. typhimurium*-challenged group with dietary supplementation of YCW; *C. perfringens*-challenged group with dietary supplementation of YCW. Each treatment was replicated 12 times. The challenge inoculums with *S. typhimurium* and *C. perfringens* were prepared according to the procedures described by Aljumaah et al. (2020a) and Hussein et al. (2020a), respectively. The YCW extract from *S. cerevisiae* (SafMannan, Phileo, Lesaffre, Marcq-en-Baroule Cedex, France) was supplemented on top of the diet at the rate of 0.5 g/kg YCW.

Sampling and measurements

Bodyweight and feed intake of each replicate were recorded every 5 d, and daily feed intake, average daily gain (ADG), feed conversion ratio (FCR), and European production efficiency factor (EPEF) were computed for each bird for the periods of 11 to 25, 26 to 35, and 11 to 35 d. The FCR was adjusted for mortality and EPEF was determined by the following formula: \(\text{viability} \times \text{body weight (kg)} / (\text{FCR} \times \text{age (d)}) \times 100\).

At d 35 of age, 1 bird per replicate was selected randomly for blood collection. Blood samples (3 mL) were obtained from the wing vein and centrifuged at 3000 rpm for 10 min to separate serum which was stored at −20°C until biochemical analysis. The concentrations of total protein, albumin, glucose, and triglycerides in the serum were measured by using commercial assay kits (Randox Laboratories Ltd., Crumlin, UK). The amount of globulin was then calculated by the difference between total protein and albumin.

After blood collection, the sampled birds were individually weighed and slaughtered and feathers, head, neck, shanks, feet, and viscera were removed. The dressing percentage was determined from eviscerated weight and pre-slaughter live weight. The remaining carcase was dissected to separate breast and thigh, and abdominal fat, liver, empty gizzard, spleen, and bursa of Fabricius were also separated. The weight percentage for each part was then calculated on the basis of pre-slaughter live weight.

The total empty weight and length of the intestine were recorded to determine the weight and length percentages of the duodenum (from the ventriculus to pancreo-biliary ducts), jejunum (from pancreo-biliary ducts to Meckel’s diverticulum), ileum (from Meckel’s diverticulum to ileocecal junction), and caecum (from ileocecal junction to the end of the hindgut), respectively.

About 1-cm section of the lower ileum was cut, washed in physiological saline solution, and fixed in 10% buffered formalin. The fixed intestinal segments were mounted onto slides after cutting up to 5-μm in thickness and the slides were then stained using haematoxylin and eosin stain as previously described (Al-Fataftah and Abdelqader 2014). The height and width of villi and crypt depth were measured based on at least 10 well-oriented crypt-villus units per section using an Olympus IX71 inverted microscope and PC-based image analysis system (Olympus DP72 microscope digital camera; Olympus NV, Aartselaar, Belgium) with software analysis (cellSens digital
imaging software for research application). The villus height to crypt depth ratio and villus surface area were then calculated (Abdelqader and Al-Fataftah 2016).

One g of the caecal digesta was aseptically emptied into a sterile test tube which was kept at 4 °C until bacterial analysis. At the laboratory, samples were diluted 1:9 (wt/vol) in sterile saline and then exposed to 10 sequential dilutions 1:9 (vol/vol) with sterile 0.85% NaCl. A 0.1-mL of each dilution was plated in duplicate on selective agar media (Oxoid, Basingstoke, Hampshire, UK) for target bacterium enumeration. For S. typhimurium isolation, Luria-Bertani agar was used with aerobic incubation at 37 °C for 24 h. The C. perfringens was cultured on tryptose sulfite-cycloserine agar and incubated under an anaerobic atmosphere at 37 °C for 48 h. Lactobacillus was isolated with de Man–Rogosa–Sharpe agar, followed by anaerobically incubation for 48 h at 37 °C. All results were expressed as log10 CFU/g of caecal content.

### Statistical analysis

Data were analysed with one-way ANOVA utilising the GLM procedure of SAS software (SAS 9.4 Institute Inc., Cary, NC). The statistical model involved treatment as a fixed factor and pen as a random factor. Differences amongst means were separated by employing Tukey’s multiple comparison test. The replication was considered as the experimental unit, and a p < .05 was regarded as statistically significant. Findings are illustrated as means and pooled SEM.

### Results

#### Performance parameters

The growth performance of broiler chickens for the grower, finisher, and overall periods is shown in Table 2. During the grower phase (11-25 d), the challenged groups without YCW had lower ADG and EPEF and higher FCR compared with the control group (p < .001). Whereas the challenged groups with YCW had improved FCR and EPEF compared with the unsupplemented challenged groups (p < .001). The improvement in FCR with YCW under S. typhimurium challenge reached the same level as the control. In the same line, broilers during the finishing stage (26-35 d) showed that the challenged groups without YCW had worsened ADG (p < .02), EPEF, and FCR (p < .001) compared with the control group, with ADG for the challenged groups fed YCW and FCR for the C. perfringens-challenged group given YCW being intermediate and did not differ from other treatments. While the challenged groups with YCW had improved EPEF compared with the unsupplemented challenged groups (p < .001). Moreover, the S. typhimurium-challenged group that received YCW had improved FCR when compared to the unsupplemented group challenged with S. typhimurium (p < .001). The improvements in FCR and EPEF by YCW during S. typhimurium challenge reached the same level as the control.

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The overall broiler growth performance from d 11 to 35 revealed that the challenged groups fed the unsupplemented diet had deteriorated ADG, FCR, EPEF, and body weight compared with the control group (p < .001), with ADG and body weight for the

| Challenge (g/kg) | Control | S. typhimurium | C. perfringens | S. typhimurium | C. perfringens | SEM | p Value |
|------------------|---------|----------------|---------------|----------------|----------------|-----|--------|
| 11-25 d          |         |                |               |                |                |     |        |
| DFI (g)          | 52.7    | 51.5           | 51.3          | 51.0           | 50.5           | 0.5 | .390   |
| ADG (g)          | 40.1    | 35.9           | 34.8          | 38.2           | 36.5           | 0.79| .001   |
| FCR (g: g)       | 1.32    | 1.44           | 1.48          | 1.34           | 1.37           | 0.67| .001   |
| EPEF (%)         | 273     | 232            | 224           | 257            | 249            | 5.81| .001   |
| 26-35 d          |         |                |               |                |                |     |        |
| DFI (g)          | 98.1    | 96.5           | 92.0          | 91.6           | 93.3           | 3.09| .510   |
| ADG (g)          | 56.5    | 48.2           | 47.5          | 52.4           | 51.4           | 2.04| .020   |
| FCR (g: g)       | 1.74    | 2.01           | 1.97          | 1.75           | 1.82           | 0.04| .001   |
| EPEF (%)         | 240     | 186            | 179           | 231            | 214            | 7.84| .001   |
| 11-35 d          |         |                |               |                |                |     |        |
| DFI (g)          | 75.9    | 74.5           | 72.6          | 72.2           | 72.5           | 1.52| .340   |
| ADG (g)          | 50.3    | 43.9           | 42.9          | 47.3           | 46.9           | 1.90| .001   |
| FCR (g: g)       | 1.51    | 1.70           | 1.70          | 1.53           | 1.58           | 0.02| .001   |
| EPEF (%)         | 276     | 224            | 215           | 266            | 250            | 7.39| .001   |
| BW (g)           | 1645    | 1535           | 1537          | 1586           | 1575           | 24.7| .030   |
| Liveability (%)  | 99.7    | 97.0           | 94.9          | 98.8           | 96.4           | 0.78| .001   |

SEM: pooled standard error of the mean. Means in the same row with different superscripts differ (p < .05).

Table 2. Daily feed intake (DFI), average daily gain (ADG), feed conversion ratio (FCR), European production efficiency factor (EPEF), and body weight (BW) of broilers fed supplemental yeast cell wall (YCW) under Salmonella typhimurium (S. typhimurium) and Clostridium perfringens (C. perfringens) challenges.
challenged groups given YCW being intermediate and not different from other treatments. On the other hand, the challenged groups given YCW had improved FCR and EPEF compared with the unsupplemented challenged groups \( (p < .001) \). The improvements in FCR of both challenged groups and EPEF of the \( S. \ typhimurium \)–challenged group due to YCW reached the same level as the control. The liveability of the unsupplemented \( C. \ perfringens \)–challenged group was lower than that of the control, but the addition of YCW makes it almost equivalent to the control \( (p < .001) \), while the unsupplemented group infected with \( S. \ typhimurium \) showed an intermediate liveability percentage between all other treatments. There was no difference \( (p > .05) \) in daily feed intake among the groups in all experimental periods.

### Carcase measurements

The results of carcase and intestinal parameters are listed in Tables 3 and 4, respectively. The carcase traits of broilers at 35 d of age unmasked that the challenged groups without YCW had a lower dressing percentage compared to the control group, with the challenged groups with YCW being intermediate and not different from any other treatment \( (p < .001) \). Whereas the other traits including the relative weights of breast, thigh, abdominal fat pad, liver, gizzard, spleen, and bursa of Fabricius were not affected by the treatments \( (p > .05) \). Similarly, all intestinal measurements were not influenced \( (p > .05) \) by either infections or feed additive except for the jejunum length percentage which was higher for the \( C. \ perfringens \) + YCW group, lower for the \( S. \ typhimurium \) + YCW group, and intermediate for the other groups \( (p < .01) \).

### Blood biochemical indexes

The effect of the treatments on serum biochemical indicators of broiler chickens at 35 d of age is shown in Table 5. The \( S. \ typhimurium \) + YCW and control groups had the highest total protein levels \( (3.19 \) and \( 3.03 \) g/dL, respectively), while the \( S. \ typhimurium \)–challenged group without YCW had the lowest total protein concentration \( (2.7 \) g/dL) \( (p < .001) \). Results also showed that the highest concentration for blood albumin was in the control and unsupplemented \( C. \ perfringens \)–challenged groups \( (1.77 \) and \( 1.75 \) g/dL, respectively) and the lowest albumin concentration was for the \( C. \ perfringens \)–challenged broilers fed YCW \( (1.53 \) g/dL), while the \( S. \ typhimurium \)–infected birds fed with or without YCW showed intermediate concentrations and did not differ from other treatments \( (p < .01) \). Broilers fed the unsupplemented diet under \( S. \ typhimurium \) or \( C. \ perfringens \) challenges had the lowest serum globulin levels \( (1.03 \) and \( 1.07 \) g/dL, respectively), but adding YCW to the diet of \( S. \ typhimurium \)–infected birds improved it \( (1.51 \) g/dL), with intermediate values for the control and \( C. \ perfringens \) + YCW groups \( (p < .05) \). Dietary supplementation of YCW increased blood glucose levels to a similar level to the control group in comparison with the unsupplemented infected chickens \( (p < .001) \). No difference in triglycerides was found between the treatments \( (p > .05) \).

### Intestinal histology

In Table 6, the observation of the histological sections of the ileum on d 35 revealed that the dietary supplementation of YCW increased villus height compared with the unsupplemented groups \( (p < .001) \). The unsupplemented challenged groups had a reduced villus surface area, which was improved by the addition of YCW to the diet of broilers challenged with \( S. \ typhimurium \), with intermediate means for the control and \( C. \ perfringens \) + YCW groups \( (p < .01) \). Crypt depth was higher for the control and \( S. \ typhimurium \) + YCW groups, lower for the \( C. \ perfringens \) + unsupplemented diet group, and intermediate for the \( S. \ typhimurium \) + unsupplemented diet and \( C. \ perfringens \) + YCW groups \( (p < .05) \). No differences in villus width

### Table 3. Carcase weights and yields as a % of pre-slaughter live weights in broilers fed supplemental yeast cell wall (YCW) under \( S. \ typhimurium \) (\( S. \ typhimurium \)) and \( C. \ perfringens \) (\( C. \ perfringens \)) challenges at 35 d.

|                | Challenge | Control | \( S. \ typhimurium \) | \( C. \ perfringens \) | \( S. \ typhimurium \) | \( C. \ perfringens \) | SEM  | \( p \) Value |
|----------------|-----------|---------|-----------------------|-----------------------|-----------------------|-----------------------|------|--------------|
| YCW (g/kg)     | 0         | 0       | 0                     | 0                     | 0.5                   | 0.5                   |      |              |
| Dressing       | 70.8\(^a\) | 69.3\(^b\) | 69.5\(^b\)           | 70.1\(^a,b\)         | 70.0\(^a,b\)         | 0.23                  | 0.001|              |
| Breast         | 37.6      | 37.3    | 35.8                  | 37.5                  | 37.3                  | 0.57                  | 0.170|              |
| Thigh          | 29.4      | 29.3    | 28.0                  | 29.9                  | 29.6                  | 0.63                  | 0.350|              |
| Fat            | 0.40      | 0.50    | 0.40                  | 0.20                  | 0.40                  | 0.08                  | 0.150|              |
| Liver          | 2.70      | 2.50    | 2.40                  | 2.80                  | 2.70                  | 0.17                  | 0.550|              |
| Gizzard        | 1.80      | 1.90    | 2.10                  | 1.80                  | 1.90                  | 0.14                  | 0.600|              |
| Spleen         | 0.07      | 0.11    | 0.09                  | 0.09                  | 0.10                  | 0.01                  | 0.310|              |
| Bursa          | 0.16      | 0.17    | 0.17                  | 0.18                  | 0.19                  | 0.01                  | 0.660|              |

SEM: pooled standard error of the mean \( (n = 12) \). Means in the same row with different superscripts differ \( (p < .05) \).
and villus height to crypt depth ratio were found between the treatments (p > .05).

**Intestinal bacterial population**

The bacterial counts from caecal contents of broilers at 35 d of age are illustrated in Table 7. Compared to the unsupplemented *S. typhimurium*-infected group, dietary supplementation with YCW during the *S. typhimurium* challenge lowered *S. typhimurium* enumeration, with no detection of *S. typhimurium* colonies in the control, *C. perfringens* without YCW, and *C. perfringens* with YCW groups (p < .01). Broilers infected with *C. perfringens* and fed the unsupplemented diet had the highest load of *C. perfringens*, but they had the intermediate level of load when YCW was supplemented, with almost no detection of *C. perfringens* colonies in broilers of other treatments (p < .01). The unsupplemented challenged groups had a lower *Lactobacillus* population as compared with the control group, with intermediate counts for the YCW-supplemented infected groups (p < .01).

**Discussion**

Yeast cell wall extract derived from *S. cerevisiae* has been widely used in broiler chickens in order to support antibiotic-free poultry (Xue et al. 2017; Pascual et al. 2020; Ahiwe et al. 2021). Several studies revealed that dietary YCW extract supplementation increased growth performance in broiler chickens through the improvements in live body weight, ADG, and FCR compared with birds fed an unsupplemented diet either in healthy birds or with intermediate levels of challenge.
challenge broilers with either *Salmonella* (Liu, Wang, Liu et al. 2018; Ahiwe et al. 2019) or *C. perfringens* (Abudabos and Yehia 2016; Johnson et al. 2020). In support of this notion, our results showed that the *S. typhimurium* or *C. perfringens*-challenged grower and finisher birds exhibited decreased ADG and EPEF and worsened FCR compared with the control group, whereas YCW supplements improved growth performance in challenged birds including better ADG, FCR, and EPEF, confirming the potential roles of YCW on restoring normal growth performance during either *S. typhimurium* or *C. perfringens* challenges. Similarly, *S. typhimurium* and *C. perfringens* challenges significantly lowered carcase dressing percentage, while the YCW-supplemented groups showed intermediate percentages with no significant difference from the control group, indicating a possible positive effect of YCW in alleviating the stress of microbial challenges on carcase yield. This result is partially in line with the findings of Ahiwe et al. (2019) who revealed that YCW heightened the dressing percentage of broiler chickens challenged with *Salmonella* lipopolysaccharides. The dietary YCW supplementation would have a trophic influence on broiler gut development (Pascual et al. 2020) as demonstrated by the increased ileum villi height and surface area observed in our study. These alterations in intestinal morphology could be associated with enhanced growth performance and carcase yield of challenged broilers as a result of increased surface area for nutrient absorption.

The analysis of blood biomarkers in broiler chickens allowed to evaluate the functional state of their body. The level of total protein in the circulations mainly reflects the synthesis or breakdown of two major proteins: albumin and globulin (Bagno et al. 2021). In the current study, the reduction in blood total protein and globulin in challenged birds with *S. typhimurium* or *C. perfringens* might be due to the induction of protein catabolism in the body, which could explain the reduction in ADG of the challenged birds. However, YCW supplementation increased total protein and globulin in the circulation, suggesting that YCW would likely upregulate protein anabolism to support better growth performance for the challenged broilers. Glucose is a crucial source of energy and controls several metabolic pathways in broiler chickens (Abasht et al. 2019). Recent studies unmasked that microbial infections would decrease blood glucose in broiler chickens. For example, *S. typhimurium* infection decreased blood glucose in finisher male broiler chickens at 35 d of age (Aljumaah et al. 2020b). Another study showed that broiler chickens challenged with *C. perfringens* had drastically decreased blood glucose level compared with unchallenged ones (Hussein et al. 2019). In support of these results, the present study reported that *S. typhimurium* or *C. perfringens* challenges decreased circulating glucose levels in the finishing broilers. However, YCW increased glucose concentrations in the infected groups to a similar level as the control group, suggesting that infection stress could be alleviated with YCW by providing extra energy to improve growth performance in the challenged birds.

Previous studies reported that microbial challenges had negative impacts on intestinal morphology in broiler chickens. For instance, Abudabos and Yehia (2016) noticed that *C. perfringens* challenge decreased ileal villus height in broiler chickens. Also, the intestine of broilers infected with *S. typhimurium* at 15 d exhibited acute desquamation of lining epithelium with extensive metaplasia of goblet cells and moderate haemorrhage between the intestinal glands (Abudabos et al. 2019). In agreement with these previous studies, histological examination for the ileum in the current study showed that challenges with *S. typhimurium* or *C. perfringens* suppressed the development of the small intestine through the reduction of villus height and villus surface area, this would lead to a poorer intestinal absorptive ability and could eventually reduce the growth performance noticed in challenged broiler chickens. Interestingly, dietary supplementation with YCW increased villus height and villus surface area, resulting in a better intestinal health status that would promote the production performance of the challenged broilers. In support of these results, a previous study reported that YCW improved villus height in chicks challenged with *C. perfringens*.

(Ahiwe et al. 2020; Pascual et al. 2020) or challenged broilers with either *Salmonella* (Liu, Wang, Liu et al. 2018; Ahiwe et al. 2019) or *C. perfringens* (Abudabos and Yehia 2016; Johnson et al. 2020). In support of this notion, our results showed that the *S. typhimurium* or *C. perfringens*-challenged grower and finisher birds exhibited decreased ADG and EPEF and worsened FCR compared with the control group, whereas YCW supplements improved growth performance in challenged birds including better ADG, FCR, and EPEF, confirming the potential roles of YCW on restoring normal growth performance during either *S. typhimurium* or *C. perfringens* challenges. Similarly, *S. typhimurium* and *C. perfringens* challenges significantly lowered carcase dressing percentage, while the YCW-supplemented groups showed intermediate percentages with no significant difference from the control group, indicating a possible positive effect of YCW in alleviating the stress of microbial challenges on carcase yield. This result is partially in line with the findings of Ahiwe et al. (2019) who revealed that YCW heightened the dressing percentage of broiler chickens challenged with *Salmonella* lipopolysaccharides. The dietary YCW supplementation would have a trophic influence on broiler gut development (Pascual et al. 2020) as demonstrated by the increased ileum villi height and surface area observed in our study. These alterations in intestinal morphology could be associated with enhanced growth performance and carcase yield of challenged broilers as a result of increased surface area for nutrient absorption.

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Table 7. Caecal bacterial counts (log_{10} CFU/g) of broilers fed supplemental yeast cell wall (YCW) under *Salmonella typhimurium* (*S. typhimurium*) and *Clostridium perfringens* (*C. perfringens*) challenges at 35 d.

| Challenge | Control | *S. typhimurium* | *C. perfringens* | *S. typhimurium* | *C. perfringens* | SEM | p Value |
|-----------|---------|-----------------|-----------------|-----------------|-----------------|-----|---------|
| YCW (g/kg) | 0       | 0.00c           | 0.00c           | 0.5             | 0.5             |     |         |
| *S. Typhimurium* | 0.00c    | 6.19a           | 0.00c           | 4.38b           | 0.00c           | 0.43| .001    |
| *C. Perfringens* | 0.00b    | 0.18b           | 4.17a           | 0.00b           | 1.83ab          | 0.79| .010    |
| *Lactobacillus* | 7.31a    | 6.80b           | 7.13ab          | 7.18ab          | 0.12            |     | .010    |

SEM: pooled standard error of the mean (*n* = 12). Means in the same row with different superscripts differ (*p < .05*).
perfringens at 16 weeks of age (Abudabos and Yehia 2016). Also, Lu et al. (2019) showed that feeding Eimeria-challenged broiler breeders and their offspring with yeast bioactives induced a longer villus height. Similarly, Johnson et al. (2020) recently noticed better intestinal functions in broilers infected with C. perfringens by feeding YCW, as demonstrated by enhanced cell growth, improved survival signalling, and decreased apoptosis that eventually reduced disease severity in the intestine.

In the present study, improved growth performance observed in the challenged chickens supplemented with YCW could be attributed to a shift in the caecal microbial community towards a healthier balance by supporting the commensal lactic acid bacteria and diminishing the detrimental bacteria including S. typhimurium or C. perfringens that might produce toxic metabolites and thus impair the development of caecum (Wilson et al. 2005). Therefore, dietary YCW could support healthy balanced intestinal bacterial communities of broiler chickens. These results agree with previous reports where Spring et al. (2000) found that dietary mannanoligosaccharides lowered caecal S. typhimurium populations, and Yang et al. (2008) demonstrated that dietary mannanoligosaccharide modulated the equilibrium of intestinal microbiota by elevating the numbers of Lactobacillus and reducing the counts of C. perfringens in the caeca of broilers.

Conclusion
Dietary supplementation with YCW could alleviate the negative effects of infection by pathogenic bacteria such as S. typhimurium or C. perfringens in grower and finisher broiler chickens probably through inducing a greater absorption capacity in the small intestine, as demonstrated in our study by stimulating longer villus height, greater villus surface area, lower colonisation of the pathogenic S. typhimurium or C. perfringens bacteria, and better colonisation of beneficial Lactobacillus bacteria. These changes would increase protein and glucose utilisation and eventually support the efficiency of growth in broiler chickens under antibiotic-free poultry production systems.

Ethical approval
The study was approved by the Research Ethics Committee, Deanship of Scientific Research, Vice-Rectorate for Graduate Studies & Scientific Research at King Saud University project approval number KSU-SE-18-38.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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ORCID
Ali R. Al Sulaiman http://orcid.org/0000-0003-4005-1569
Alaeldein M. Abudabos http://orcid.org/0000-0001-7998-0344

Data availability statement
The data that support the findings of this study are available from the corresponding author, [Abudabos AM], upon reasonable request.

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