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# Effect of Prebiotic Yeast Cell Wall on Starter Broilers Undergoing Gastrointestinal Challenge

**Raghad A Abdaljaleel[*1,2], Alsadwi AM[1,2], Morouj M Al-Ajeeli[1], Hector Leyva-Jimenez[1], James A Byrd[3], Christopher A Bailey[1]**

*[1]Department of Poultry Science, Texas A&M University, College Station, TX 77843 2472, USA, [2]Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, [3]USDA Southern Plains Agriculture Research Center, College Station, TX 77843-2480, USA*

***Correspondence**:
raghad@covm.uobaghdad.edu.iq

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## A B S T R A C T

In recent years, yeast cell wall (YCW) prebiotics have been increasingly explored as alternatives to prophylactic antibiotic growth promoters in poultry production. Threonine, the third limiting amino acid in broiler diets, plays an essential role in protein synthesis and gut health. This study evaluated the effects of dietary YCW supplementation on ileal amino acid digestibility, intestinal morphology, and performance of starter broilers fed a marginal threonine-to-lysine ratio (0.60) under gastrointestinal challenge conditions. A total of 240 one-day-old Ross 308 broiler chicks were used in a 21-day experiment. Birds were allocated to 48 pens, with 5 birds per pen, and assigned to four dietary treatments. A basal diet containing 22% crude protein, 2980 kcal/kg metabolizable energy, 1.35% calculated lysine, and 0.81% calculated threonine was prepared and supplemented with or without YCW at 250 ppm. To induce a gastrointestinal immune challenge, birds were vaccinated at 10 days of age with a commercial infectious bursal disease vaccine at 10 times the recommended dose, followed by oral challenge with *Clostridium perfringens* on days 16 and 17. Growth performance was assessed on days 10, 16, and 21. On day 21, ileal digesta were collected for amino acid digestibility analysis, and jejunal and ileal tissues were sampled for histomorphological measurements. Supplementation with 250 ppm YCW significantly improved body weight gain, feed conversion ratio, and productivity index before challenge and after infectious bursal disease vaccination. After *C. perfringens* challenge, a significant interaction was observed for productivity index, with the highest value recorded in non-challenged birds receiving YCW. Overall, YCW supplementation improved the productivity index of broilers fed a low threonine-to-lysine ratio, mainly through reduced mortality. However, it did not significantly influence ileal amino acid digestibility, intestinal morphology, or necrotic enteritis lesion scores.

**Keywords:** prebiotic yeast cell wall, *Clostridium perfringens*, threonine, lysine, broiler

## INTRODUCTION

Enteric diseases are an important concern to the poultry industry as they can affect productivity by increasing mortality and causing contamination of poultry products for human consumption (**1**). In poultry, one of the most causes of necrotic enteritis is *Clostridium perfringens* (*C. perfringens*) (**2**). In addition to necrotic enteritis, *C. perfringens* can also cause necrotic dermatitis, ulcerative enteritis, necrotic hepatitis and botulism. These diseases can occur when a high frequency of adhesion by *C. perfringens* causes damage to the intestinal mucosa (**3**).

*C. perfringens* is often present naturally in high amounts in poultry environments (**4**).

Bacterial resistance to antibiotics has increased interest in eliminating sub-therapeutic use of antibiotics in livestock. Antimicrobial feed additives such as probiotics, prebiotics and symbiotics are dietary supplements that can enhance the health of host and develop the performance of animals through different mechanisms (**5,6**). In poultry, both probiotics and prebiotics can help reduce enteric disease (**1,7**). Prebiotics are non-digestible carbohydrates with selective impact on intestinal bacteria and immunity of chickens (**8,9**). Commercial prebiotics can be derived from the outer layer of Yeast Cell Wall (YCW) and contain a mixture of mannano-oligosaccaridi (MOS) with ß-glucan and mannoprotein (**10**). Many older publications have described YCW products as MOS; which technically correct since the MOS only represents a fraction of the entire YCW. Yeast cell wall products that are derived from *Saccharomyces cerevisiae* can improve intestinal mucosa through increasing villi height and goblet cell density, as well as improving the productivity of the birds (**11,12**).

The supplementation of essential amino acids in least cost poultry diets is important with threonine often considered as the third limiting amino acid after methionine and lysine in typical broiler diets (**13**). Ileal digestibility is currently a preferred approach to estimate amino acid availability in feed ingredients. The assay for standardized ileal digestibility eliminates some of the shortcomings of fecal digestibility, and a standardized correction can be made for basal endogenous losses (**14**). Data sets for establishment of the standardized digestibility system in broiler feeding were published by (**14**), who stated that the optimum standardized ileal digestibility for threonine in broiler chicks at days 1-5, 6-14 and 15-35 days of age are 0.82, 0.80, and 0.72%, respectively.

Although, there has been much research that focuses either on threonine requirements or on YCW alone, with respect to their effects on growth performance, morphology of intestine, and ileal digestibility, there is only limited research that addresses whether or not YCW affects threonine requirements and none that we know of using a *C. perfringens* challenge model. The objective of this study was to evaluate the effects of a prebiotic YCW on performance, intestinal morphology and ileal digestibility of young broilers fed diets with a marginal threonine to lysine ratio of 0.60 while undergoing a Gastro Intestinal (GI) challenge. The hypothesis being that YCW will increase ileal threonine digestibility improving broiler performance while reducing the overall threonine requirement in birds subjected to a GI immune challenge.

## Materials And Methods

### Ethical Approval

This study was performed at the Texas A&M University Poultry Research Center, following protocols approved by the Texas A&M institutional Animal Care and Use Committee (IACUC 2014-0030) and the Animal Care and Use Committee at the Southern Plains Agriculture Research Center. Birds were housed at the Southern Plains Agricultural Research Center; United States Departments of Agriculture.

### Experimental Design and General Procedures

A randomized complete block design arranged as a 2×2 factorial was used throughout the study to determine bird's performance. The non-dependent variables were the presence or absence of dietary YCW in birds either challenged or non-challenged with *C. perfringens*. A total of 240 Ross 308 straight-run broiler chicks were distributed among 2 Petersime battery brooder units. A total of 4 treatment groups with 12 replicates for each treatment were randomly assigned to pens, with 48 individual pens used for housing with 5 birds in each pen. Birds were fed an industry-type basal broiler starter corn-soy diet with 2980 kcal/kg ME and 22% protein. The calculated amount of threonine used in the diet was 0.81% while the calculated lysine was 1.35%, resulting in a threonine to lysine ratio of 0.60 with and without YCW at 250 ppm. Feed and water were offered to birds ad libitum. Diet composition is described in **Table 1**.

| Ingredient | Percentage Diet |
|---|---|
| Yellow corn | 63.5 |
| Dehulled soybean meal | 31.4 |
| DL-Methionine | 0.28 |
| Lysine HCL | 0.26 |
| Soybean oil | 0.79 |
| Limestone | 1.44 |
| Mono dicalcium $PO_4$ | 1.55 |
| Salts | 0.51 |
| Vitamins premix[1] | 0.25 |
| Trace Minerals premix[2] | 0.05 |
| **Calculated Nutrient Content (%)** | |
| Metabolizable Energy (Kcal/kg) | 2980 |
| Protein | 22.0 |
| Crude Fat | 2.68 |
| Crude Fiber | 2.15 |
| Available Phosphate | 0.45 |
| Calcium | 0.95 |
| Methionine | 0.61 |
| Methionine + Cystine | 0.97 |
| Lysine | 1.35 |
| Threonine[3] | 0.81 |
| Tryptophan | 0.25 |

[1]Vitimin premix provided the following, per kilogram of diet: vitamin A 11 KIU; vitamin $D_3$, 3,850 IU; vitamin E, 45.8 IU; vitamin $B_{12}$, 0.017 mg; biotin, 0.55 mg; menadione, 1.5 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; vitamin $B_6$, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg. [2]Trace minerals provided in the following, per kg of diet: Cu, 7.0 mg; I, 0.4 mg; Fe, 60.0 mg; Mn, 60.0 mg; Zn, 60.0 mg. L-threonine (98%) was added at 0.07 to create calculated threonine concentration of 0.81. The assayed concentration was 0.74. * The prebiotic used for this experiment was a yeast cell wall (YCW) derived from *Saccharomyces cerevisiae*, SafMannan (Phileo-Lesaffre Animal Care, Milwaukee, WI, USA)

The environment of the rearing room was completely controlled, and 24-h lighting was provided. Birds were weighed by pen, and the body weight and feed intake was recorded at 10, 16 and 21 day of the experimental period. A commercial Infectious Bursal Disease (IBD) vaccine (Schering-Plough Animal Health, Millshoro, DE) was used for all birds at 10× the manufacture's recommended dose to compromise the humoral immune system at day 10 of the study followed by *C. perfringens* challenge for the challenged groups (24 of the 48 available pens) on days 16 and 17. The *C. perfringens* were field isolates (Georgia and Texas combined cultures) known to cause necrotic enteritis (**15**). Every bird received the *C. perfringens* challenge (3 mL) with $10^7$ CFU of *C. perfringens*/mL administered by oral gavage.

The performances variable evaluated in this study were body weight (BW), weight gain per bird (WG), feed conversion ratio (FCR), productivity index (PI) and mortality. The following mathematical formula was used in order to calculate the PI:

PI = (100-Mortality) × (BW/1000)/Bird age/FCR×100

## Histology Samples Preparation

At day 21 of the experiment, one bird per pen was selected randomly and samples from jejunum and ileum were collected for evaluation of histological morphology. Thus, 12 birds from each treatment were evaluated. Approximately 3 cm lengths of the small intestine were collected: jejunum (about 2 cm proximal of the Meckel's diverticulum towards the distal duodenal loop); and ileum (about 2 cm proximal of the ileocecal junction towards Meckel's diverticulum). The intestinal segments were washed, prepared, processed and stained with hematoxylin and eosin stain (StatLab, Mckinney, TX). Histological sections were examined with a Nikon Super Cool Scan 5000 (Nikon, Inc., Melville). Five well-oriented villi for each replicate from jejunal and ileal sections were measured. The variables that measured were villi height (VH), crypt depth (CD), villi height over crypt depth ratio (VH/CD), villi width (VW) and the muscularis thickness (MST). ImageJ software (ImageJ, National Institutes of Health, Bethesda, MD) was used for measurement.

## Lesion Scoring for Necrotic Enteritis

Postmortem examination for necrotic enteritis lesions was performed on the small intestine at day 21 of the study. A cervical dislocation was used to kill 4 birds from each pen and signs of necrotic were examined by using 0-4 scoring system of sacrificed birds by veterinarians in the Southern Plains Agricultural Research Center. The necrotic enteritis lesion scores were as follows: 0 = no lesion, normal healthy intestine, 1= thin small friable in small intestine, gray appearance, 2 = ulceration, focal necrosis, gray appearance and thin wall, 3 = small hemorrhage with visible gas production in small intestine and sizable patchy necrosis, 4 = severe extensive necrosis with large hemorrhage, large amounts of gas in the small intestine (**16**).

## Standardized Amino Acid Ileal Digestible Coefficient

An indigestible marker (Titanium dioxide) was added at the rate of 5 g kg-1 to the experimental diet. One bird at 21 d of age was kept in each pen for amino acids analysis. Birds consumed feed that contained titanium dioxide for 22 and 23-days of the study ileum contents from one bird per pen were collected with a total of 12 for each treatment. The samples of Ileal digesta were obtained from the lower half of the ileum, about 2 cm above the ileal-cecal junction towards Meckel's diverticulum. The tubes were immediately stored at -20°C, freeze-dried and ground for further analysis using a coffee grinder (Mr. Coffee, Sunbeam Products Inc., Boca Raton, FL). Samples from feed were also collected from each treatment for analysis. To obtain a sufficient amount of ileal digesta for the analysis, samples of 4 pens per treatment were combined, and that gave us a total of 3 replicates per treatment. Both ileal digesta and feed samples were submitted to a laboratory (Agriculture Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO) for amino acid analysis. Apparent ileal Digestibility coefficients (AaDC) of amino acids were used to calculate the standardized ileal digestibility by correcting for the basal endogenous amino acid losses according to (**14**).

## Statistical Analysis

All performance data from d 0–16 were analyzed as one way ANOVA using the 6 pen levels (height from the floor; 2 reps of all treatment per level), while performance and histology data for d 16-21 were analyzed as 2×2 factorials using the GLM procedure of SPSSTM (IBM, Armonk, NY, USA) for the four treatments with the main effect for the challenge or non-challenge and presence or absence of YCW, as well as interaction and block effect. Means were separated using Duncan's multiple range tests at *P*-value ≤ 0.05 unless otherwise noted.

# RESULTS AND DISCUSSION

## Growth Performance

In the present study diet was formulated to contain the calculated threonine to lysine ratio of 0.60 with and without YCW at 250 ppm. Half the birds were challenged with *C. perfringens* and half were not. The results showed that the YCW significantly improved BW per bird, WG, FCR and PI compared with the control group not receiving YCW at 10 and 16-days of the experiment, before *C. perfringens* challenge (**Table 2**).

This is in contrast with the results of (**17**), who stated that before *C. perfringens* challenge, from 0-14 d of feed intake, BWG and FCR were not affected by adding YCW at 300 ppm for broiler chicks in a study that evaluated the effect of yeast derived product and antibiotics on growth performance with *C. perfringens* challenge. Other than the standard vaccinations received at the hatchery the 10 day old birds did not receive any purposeful stress to the immune system. The birds did not receive a hatchery coccidiosis vaccine prior to beginning the experiment. Weight gain at 10 days averaged 218 g for birds receiving the YCW prebiotic which was 4% higher than the control group not receiving YCW. Feed conversion averaged 1.21 for birds receiving the prebiotic versus 1.24 for the control group. Performance index increased 7.6% from 198 to 213. All birds received an ocular IBD vaccination at 10x the manufacturers recommended dosage on day 10 of the experiment to stress the humoral immune system. During this phase, performance gains continued to increase in a similar fashion to that observed in the 10-day old birds (**Table 2**). It is generally recognized that yeast derived products can have beneficial effects on performance, but this result is not always seen in non-stressed birds.

However, for day 16-21 of the study there were significant main effects for the non-challenged group with lowest mortality and best phase FCR. In addition, an interaction between the challenge and YCW groups at day 21 was found for the PI. Due to this interaction, the results for the PI were analyzed with a one-way ANOVA. The PI for the non-challenged birds fed YCW was higher and significantly different from other treatments. Additionally, Mortality averaged 7.5% in the challenged birds versus 0.83% for the unchallenged broilers (**Table 3**). Feed conversion ratio increased from 1.56 in non-challenged birds to 1.73 for the challenged broilers.

**Table 2.** Effects of yeast cell wall (YCW) on performance of pre-challenge 10-d-old and IBD vaccine challenged 16-day-old of broiler chicks

| | **Inclusion of YCW ppm[5]** | |
|---|---|---|
| | **0** | **250** |
| **YCW Main Effect** | | |
| BW (g)[1] | | |
| Day 0-10 pre–IBD Vaccination | 249±3.00 [b] | 258±3.00 [a] |
| Day 16 post–IBD Vaccination | 528±6.00 [b] | 549±7.00 [a] |
| WG (g)[2] | | |
| Day 1-10 pre–*Clostridium*-challenge | 209±3.00 [b] | 218±3.00 [a] |
| Day 10-16 IBD vaccine challenge | 279±3.00 [b] | 290±5.00 [a] |
| FCR[3] | | |
| Day 0-10 pre–*Clostridium*-challenge | 1.24±0.01 [b] | 1.21±0.01 [a] |
| Day 10-16 IBD vaccine challenge | 1.42±0.01 [b] | 1.39±0.01 [a] |
| PI[4] | | |
| Day 0-10 pre–*Clostridium* challenge | 198±4.00 [b] | 213±2.00 [a] |
| Day 16 IBD vaccine challenge | 240±4.00 [b] | 258±4.00 [a] |
| Mortality (%) | | |
| Day 0-10 pre–*Clostridium* challenge | 1.67±1.00 | 0.83±0.80 |
| Day 10-16 IBD vaccine challenge | 0.00±0.00 | 0.83±0.83 |

Values are mean±SEM. [a,b] means within a row for the specified variable within no common superscript differ ($P \leq 0.05$). [1]Body weight, [2]Weight gain, [3]Feed conversion ratio (g feed consumed/g weight gained), [4]Productivity Index = (100-Mortality) × (BW/1000)/Bird age/FCR×100. [5]Main effect means represent the average of 24 pens per treatment

**Table 3.** Effects of yeast cell wall (YCW) on performance of *C. perfringens* challenge 21 d old broiler chicks

| ***C. perfringens*** | **Inclusion of YCW ppm** | | |
|---|---|---|---|
| | **0** | **250** | **Main Effect Challenge[5]** |
| BW (g)[1] | | | |
| Non-Challenge | 825±13.0 | 850±19.0 | 838±12.0 |
| Challenge | 819±23.0 | 819±17.0 | 819±14.0 |
| **Main Effect YCW** | 822±13.0 | 834±13.0 | |
| 16-21d WG (g)[2] | | | |
| Non-Challenge | 302±8.00 | 298±13.0 | 300±8.00 |
| Challenge | 287±18.0 | 272±13.0 | 279±11.0 |
| **Main** Effect **YCW** | 294±10.0 | 286±9.00 | |
| 16-21 d FCR[3] | | | |
| Non-Challenge | 1.59±0.02 | 1.54±0.02 | 1.56±0.02 [a] |
| Challenge | 1.64±0.01 | 1.82±0.01 | 1.73±0.01 [b] |
| **Main Effect YCW** | 1.62±0.01 | 1.68±0.02 | |
| PI[4] | | | |
| Non-Challenge | 264±7.00 [b] | 295±11.0 [a] | 279±7.00 |
| Challenge | 252±12.0 [cb] | 230±9.00 [c] | 241±8.00 |
| **Main** Effect **YCW** | 258±7.00 | 263±10.0 | |
| Mortality (%) | | | |
| Non-Challenge | 1.67±1.70 | 0.00±0.00 | 0.83±0.83 [a] |
| Challenge | 5.00±3.60 | 10.0±3.01 | 7.50±2.34 [b] |
| **Main** Effect **YCW** | 3.33±0.68 | 5.00±1.80 | |

Values are mean±SEM, n=12. [a-c] Main effect means within a row for the specified variable with no common superscript differ significantly ($P \leq 0.05$). [1]Body weight. [2]16-21 d weight gain. [3]16-21 d feed conversion ratio (g feed consumed/g weight gained). [4]Productivity index= (100-Mortality) × (BW/1000)/Bird age/FCR×100. [5]Main effect means represent the average of 24 pens per treatment

YCW components have been reported to improve intestinal health due to anti-pathogenic effects (**18-20**). Yeast cell wall can also improve gut health and provide favorable conditions for beneficial intestinal bacteria such as Bifidobacterium and Lactobacillus spp. as well occupying competitive binding sites for pathogenic bacteria (**21-23**).

A study conducted by (**24**) which study the growth effects of supplemented of different concentrations and sources of prebiotic YCW products using a methodology similar to the current study. This study concluded that feeding birds YCW increased growth and improved FCR compared with the control group in immune stress and challenge with *C. perfringens*, in addition to the optimal level of prebiotic YCW in starter broilers was approximately 250 to 300 ppm.

## Intestinal Morphology

The intestinal morphological results for both jejunum and ileum showed that there were no significant differences between all intestinal variables measured at d 21 of the study (**Table 4**). A number of studies have observed greater VH and superior ileal mucosa development in chickens by using YCW products prepared from *Saccharomyces cerevisiae*, especially in the first week of chicken life (**12,25**). There is less information in the literature explaining the effect of YCW on the intestinal histology of broilers in the presence of *C. perfringens* challenge. A study conducted by (**26**) studied the effect of dietary YCW (SafMannan®) (S.I. LeSaffre, Marcq en Baroeul, France) on the intestinal morphology of broilers in the presence of *C. perfringens* challenge. The results of the current experiment showed that adding YCW at 0.5% g/kg helps to better maintain ileal VH compared with a 0.01 g/kg enramycin treatment in broilers at day 16 under *C. perfringens* challenge.

**Table 4.** Effects of *C. perfringens* challenge and YCW on jejunum and ileum mucosa at 21 d old broiler chicks

| ***C. perfringens*** | **Inclusion of YCW ppm** | | |
|---|---|---|---|
| | **0** | **250** | **Main Effect Challenge[5]** |
| JVH (μm)[1] | | | |
| Non-Challenge | 758±27 | 712±40 | 735±40 |
| Challenge | 751±42 | 736±36 | 743±27 |
| **YCW Main Effect** | 754±24 | 724±27 | |
| IVH (μm)[2] | | | |
| Non-Challenge | 418±14 | 433±17 | 425±11 |
| Challenge | 390±20 | 433±18 | 412±14 |
| **YCW Main Effect** | 404±12 | 433±12 | |
| JCD (μm)[3] | | | |
| Non-Challenge | 79±2.0 | 78±3.0 | 78±2.0 |
| Challenge | 82±4.0 | 89±9.0 | 85±5.0 |
| **Main Effect YCW** | 80±2.0 | 83±5.0 | |
| ICD (μm)[4] | | | |
| Non-Challenge | 58±2.0 | 61±3.0 | 59±2.0 |
| Challenge | 62±4.0 | 72±7.0 | 67±4.0 |
| **Main Effect YCW** | 60±2.0 | 67±4.0 | |
| JVH/CD[5] | | | |
| Non-Challenge | 9.7±0.5 | 9.2±0.5 | 9.5±0.3 |
| Challenged | 9.6±0.7 | 9.3±0.9 | 9.4±0.6 |
| **Main Effect YCW** | 9.7±0.4 | 9.2±0.5 | |
| IVH/CD[6] | | | |
| Non-Challenge | 7.3±0.4 | 7.3±0.1 | 7.3±0.3 |
| Challenge | 6.6±0.2 | 6.5±0.5 | 6.6±0.4 |
| **Main Effect YCW** | 6.9±0.4 | 6.9±0.3 | |
| JVW (μm)[7] | | | |
| Non-Challenge | 90±7.0 | 93±6.0 | 91±4.0 |
| Challenge | 89±3.0 | 91±6.0 | 90±4.0 |
| **Main Effect YCW** | 90±4.0 | 92±4.0 | |
| IVW (μm)[8] | | | |
| Non-Challenge | 80±4.0 | 77±5.0 | 78±3.0 |
| Challenge | 91±7.0 | 87±5.0 | 89±4.0 |
| **Main Effect YCW** | 85±4.0 | 82±4.0 | |
| JMST[9] | | | |
| Non-Challenge | 106±5.0 | 104±7.0 | 105±4.0 |
| Challenge | 108±3.0 | 116±6.0 | 112±3.0 |
| **Main Effect YCW** | 107±3.0 | 110±4.0 | |
| IMST[10] | | | |
| Non-Challenge | 96±4.0 | 99±5.0 | 97±3.0 |
| Challenge | 101±6.0 | 108±5.0 | 105±4.0 |
| **Main Effect YCW** | 99±4.0 | 104±4.0 | |

Values are mean±SEM, n=12. [1]Jejunum villi height (μm). [2]Ileum villi height (μm). [3]Jejunum crypt depths (μm). [4]Ileum crypt depths (μm).[5]Jejunum villi height / crypt depth, [6]Ileum villi height/crypt depth. [7]Jejunum villi width (μm). [8]Ileum villi width (μm). [9]Jejunum muscularis thicknesses (μm). [10]Ileum muscularis thicknesses (μm)

## Severity of Lesions

The severity of necrotic enteritis lesions at day 21 of the experiment is given in (**Table 5**). *C. perfringens* challenge significantly increased average lesion scores from 0.62 to 1.05. Main effect means for dietary YCW were not significantly different from each other. Alizadeh et al. (**17**) performed a study to explore the effect of yeast-derived products fed at 300 ppm on performance and gut lesion scores of broilers challenged with *C. perfringens*. Their results concluded that there were no significant differences in lesion scores between the YCW challenged treatment and the control challenged and non-challenged treatments at 21 days of age which is in agreement with our results. The lack of positive effects of the YCW for minimizing the necrotic enteritis lesions may be due to the lack of their antibacterial activity against *C. perfringens* as opposed to their cell wall polysaccharides being effective against bacteria with type-1 fimbriae such as Salmonella (**27**).

**Tableable 5.** The severity of the necrotic enteritis lesions in 21 d old broiler chicks after subclinical *C. perfringens* infection

| | Inclusion of YCW ppm | | |
|---|---|---|---|
| | 0 | 250 | Main Effect Challenge[5] |
| ***C. perfringens*** | | | |
| Non-Challenge | 0.58±0.02 | 0.67±0.02 | 0.62±0.01 [b] |
| Challenge | 0.97±0.04 | 1.12±0.03 | 1.05±0.02 [a] |
| **YCW Main Effect** | 0.78±0.02 | 0.89±0.01 | |

Values are mean±SEM, n=12. [a,b] Main effect means within a column for the specified variable with no common superscript

**Table 6.** Effects of challenge and YCW on standardized ileal digestibility in 21 d old broilers chicks

| *C. perfringens* | Inclusion of YCW ppm | | |
|---|---|---|---|
| | 0 | 250 | Main Effect Challenge[5] |
| Threonine | | | |
| Non-Challenge | 83.2±1.1 | 81.4±1.1 | 82.3±0.8 |
| Challenge | 83.7±0.4 | 81.6±0.9 | 82.6±0.5 |
| **YCW Main Effect** | 83.4±0.6 | 81.5±0.7 | |
| Methionine | | | |
| Non-Challenge | 93.2±0.8 | 94.9±0.7 | 94.1±0.5 |
| Challenge | 93.9±0.7 | 94.8±0.5 | 94.4±0.4 |
| **YCW Main Effect** | 93.6±0.5 | 94.8±0.4 | |
| Lysine | | | |
| Non-Challenge | 90.1±0.6 | 90.2±0.7 | 90.1±0.4 |
| Challenge | 90.5±0.4 | 88.8±0.5 | 89.6±0.3 |
| **YCW Main Effect** | 90.3±0.3 | 89.5±0.4 | |
| Histidine | | | |
| Non-Challenge | 91.0±0.7 | 90.6±0.9 | 90.8±0.5 |
| Challenge | 91.8±0.4 | 89.6±0.4 | 90.7±0.4 |
| **YCW Main Effect** | 91.4±0.4 | 90.1±0.5 | |
| Leucine | | | |
| Non-Challenge | 87.3±0.9 | 85.8±1.2 | 86.6±0.7 |
| Challenge | 87.4±0.4 | 85.4±0.2 | 86.4±0.3 |
| **YCW Main Effect** | 87.4±0.4 | 85.6±0.5 | |
| Isoleucine | | | |
| Non-Challenge | 87.9±0.9 | 87.2±1.1 | 87.6±0.7 |
| Challenge | 88.2±0.4 | 86.2±0.4 | 87.2±0.3 |
| **YCW Main Effect** | 88.1±0.5 | 86.7±0.6 | |
| Valine | | | |
| Non-Challenge | 84.2±0.5 | 83.2±1.1 | 83.7±0.7 |
| Challenge | 84.8±0.5 | 82.6±0.6 | 83.7±0.4 |
| **YCW Main Effect** | 84.5±0.6 | 82.9±0.6 | |
| Phenylalanine | | | |
| Non-Challenge | 87.7±0.8 | 86.5±1.1 | 87.1±0.7 |
| Challenge | 88.1±0.4 | 85.7±1.2 | 86.9±0.3 |
| **YCW Main Effect** | 87.9±0.4 | 86.1±0.5 | |

Values are mean±SEM, n=3

## Ileal Digestibility

The standardized ileal digestibility coefficients for different amino acids are shown in (**Table 6**). In general, there were no significant differences in ileal digestibility observed for this study. This was unexpected given the significant increase in lesion scores observed in the *C. perfringens* challenged birds. Perhaps this group of surviving birds used to determine ileal digestibility 7 d after their initial exposure to *Clostridia* were able to resist the infection. Unfortunately, this group of birds was not scored for necrotic enteritis lesions. This is in agreement with a study by (**28**) who reported that neither hydrolyzed yeast nor level of dietary threonine had a significant effect on standardized or apparent ileal digestibility of lysine or methionine. In a study conducted by (**29**) apparent amino acid digestibility improved at 21 days of age compared with 3 to 4 days for chicks fed 4 g/kg (4000 ppm) of hydrolyzed yeast. A study done by (**24**) suggested that adding too much YCW can negatively affect performance, it was suggested the optimal concentration of YCW was between 250-300 ppm. A study was done by (**30**) investigated the dose response of threonine supplementation and to validate the threonine requirements for 1-d-old Ross 308 male broilers using a subclinical *Clostridium* infection model with different standardized digestible threonine to lysine ratios. The results indicated that the BWG and feed intake were highest for infected birds with a digestible threonine to lysine ratio of 0.67 than 0.63.

In conclusion, adding the prebiotic (YCW) at 250 ppm into broiler diets with a "low" threonine to lysine ratio of 0.60 improved PI of starter broilers not challenged with *C. perfringens*, primarily due to reduced mortality. However, the prebiotic YCW did not significantly affect ileal amino acid digestibility or reduce necrotic enteritis lesion scores

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## Conflict of interest

The authors declare no conflict of interest.

## Editorial Transparency

Akhil M Alsadwi serves as an Editorial Manager for The Iraqi Journal of Veterinary Medicine. Despite this role, the peer review process and the final publication decision were made independently and impartially, ensuring no influence from the author's editorial position.

## Author Contributions

Conceptualization, Methodology, Resources: R.A.A., C.A.B., J.A.B.; Investigation (experiments and sample collection): R.A.A., A.M.A., M.M.A., H.L.; Data Curation, Formal Analysis: R.A.A., H.L.; Writing – Original Draft: R.A.A.; Writing – Review & Editing: C.A.B., A.M.A, H.L. All authors have read and approved the final version of the manuscript.

## Artificial Intelligent Declaration

The authors declare that they are responsible for the accuracy and integrity of all content of the manuscript, including part generated by AI, and it is not used as a co-author.

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