Growth promoting effects of prebiotic yeast cell wall products in starter broilers under an immune stress and *Clostridium perfringens* challenge

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Primary Audience: Nutritionists, Production Managers, Researchers

SUMMARY

This study was designed to investigate the growth promoting effects of supplementing different sources and concentrations of prebiotic yeast cell wall (YCW) products containing mannanoligosaccharides in starter broilers under an immune stress and *Clostridium perfringens* challenge. Through a series of 6 individual studies either 240 or 288 newly hatched chicks were randomly distributed to specific dietary treatments. All birds received a commercial infectious bursa disease vaccine at 10 or 15 d age followed by an orally administered *Clostridium perfringens* challenge on d 15, 16, and 17; or 18, 19, and 20. Weekly BW, feed consumption, and daily mortality were recorded per pen. Each study was terminated after 21 d. Pooled data analysis of all studies revealed no effect between different product sources of YCW. Products from both sources produced a significant improvement in growth rate compared to control birds fed no YCW. However, a blend of two YCW products showed an approximate 15% improvement in growth rate and a 10% reduction in FCR. The optimum dose of any YCW product among those tested was determined to be approximately 250 ppm. Prebiotic YCW additives increased BW and improved feed conversion and may be considered as alternative growth promoters for starting broilers.

Key words: yeast cell wall, mannanoligosaccharide, broiler, prebiotic, growth promotion

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DESCRIPTION OF THE PROBLEM

Antimicrobial feed additives have been shown to have a tremendous effect on growth rate, feed efficiency, and reducing the colonization of enteric pathogens [1, 2]. Regulations on the prophylactic use of antibiotic growth promoters in the European Union have accelerated research into finding alternate strategies to improve animal health and promote growth in recent years. Prebiotic feed additives are one possible strategy of enhancing animal health and improving productive performance in the absence of antibiotic growth promoters. Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively inducing the growth and/or activity of one or a limited number of bacterial species already resident in the colon and thus improve health [3]. Prebiotic mannanoligosaccharides (**MOS**) can be derived from the outer layer of yeast cell walls (**YCW**), which contain a mix of the

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oligosaccharides along with β-glucans and mannoproteins [4, 5]. Therefore the practice of describing any YCW product as "MOS" is not entirely correct. The MOS present in YCW can prevent pathogenic bacteria that contain Type-1 fimbriae with mannose-seeking lectins [*Escherichia coli* (*E. coli*) and *Salmonella* spp.] from adhering to and colonizing the intestine [6]. The inclusion of YCW components from *Saccharomyces cerevisiae* (*S. cerevisiae*) can also lead to favorable direct effects on the intestinal mucosa itself (e.g., increasing villi height and goblet cell density) and an improvement in animal productivity [7, 8].

Broilers encounter a variety of stress factors in a commercial environment. The application of results from well-controlled research trials conducted under experimental conditions may not always be necessarily applicable in commercial settings. In this study, broiler performance was evaluated under an experimentally induced immune stress and pathogen-challenge condition. A series of 6 studies were conducted to evaluate the effects of different sources and concentrations of YCW products on the performance of starter broilers subjected to a live-attenuated vaccine against infectious bursal disease (**IBD**) followed by a *Clostridium perfringens* (*C. perfringens*) challenge. We hypothesized that the supplementation of YCW improves performance under these stress conditions and that the effectiveness depends on both the source and concentration of YCW.

MATERIALS AND METHODS

Six trials were conducted to investigate different prebiotic YCW products: Safmannan (derived from baker's yeast), Pronady (derived from brewer's yeast), and Biosaf (a heat-stable concentrate of live *S. cerevisiae* yeast). All yeast products evaluated in this study were provided by LeSaffre Feed Additives (Milwaukee, WI). All rearing methods were approved by the Texas A&M Institutional Animal Care and Use Committee.

General Procedure for All Studies

Common procedures followed in all studies are explained here with specific differences given under each study subheading. Straight-run, Ross-308 broiler chicks were obtained from a commercial hatchery. All feed and water were offered *ad libitum* with continuous lighting. A basal, corn/soy-based broiler starter diet was prepared (Table 1). The basal diet was divided into equal-sized batches depending on the number of dietary treatments in that particular study, and each batch was supplemented with one of the YCW products at a specific concentration. Dietary treatments were randomly assigned to pens such that each treatment was presented at least once for any given vertical row of pens within the battery brooder. Daily observations were made with regard to general flock condition, temperature, lighting, water, feed, and unanticipated events in the house.

Performance variables evaluated in this study were final BW per bird, phase weight gain (**WG**) per bird, total feed consumption (**FC**),

Table 1. Composition and nutrient content of the basal broiler starter diet used throughout this study.

Ingredient	Amount (%)
Corn	58.4
Dehulled soybean meal	34.5
DL-Methionine 98%	0.23
Lysine HCl	0.18
Fat, blended animal/vegetable	2.76
Limestone	1.56
Mono-dicalcium phosphate	1.54
Salt	0.51
Trace minerals premix[1]	0.05
Vitamin premix[2]	0.25
Calculated nutrient content (%)	
CP	22.0
ME (Kcal/kg)	3050
Crude fat	5.32
Crude fiber	2.63
Calcium	0.95
Available phosphate	0.71
Sodium	0.22
Methionine	0.56
Lysine	1.31

[1]Trace minerals premix added at this rate yields (mg/kg): zinc, 60.0; manganese, 60.0; iron, 60.0; copper, 7.0; iodine, 0.4.

[2]Vitamin premix added at this rate yields (per kg): vitamin A, 11 kIU; vitamin D_3, 3,850 IU; vitamin E, 45.8 IU; menadione, 1.5 mg; B_{12}, 0.017 mg; biotin, 0.55 mg; thiamine, 2.93 mg; riboflavin, 5.96 mg; d-pantothenic acid, 20.17 mg; B_6, 7.15 mg; niacin, 45.8 mg; folic acid, 1.74 mg; choline, 130.3 mg.

Table 2. Study design showing the number of treatments, birds, and vaccination/challenge schedule throughout this study.

Study	Treatments[1]	Birds/pen	Vaccination[2]	*C. perfringens* challenge
Study 1	9	5	d 15	d 18, 19, and 20
Study 2	6	5	d 15	d 18, 19, and 20
Study 3	9	5	d 15	d 18, 19, and 20
Study 4	8	5	d 15	d 18, 19, and 20
Study 5	8	6	d 10	d 16, 17, and 18
Study 6	8	6	d 10	d 16, 17, and 18

[1]The total number of dietary treatments for that particular study.

[2]Birds in Study 1 received Cocci-Vac applied to the feed and birds in all remaining studies received a live-attenuated IBD vaccine via ocular route.

FCR, productivity index (**PI**), and percent mortality (**MORT**). PI was calculated using the following formula: livability [%]×BW [kg]/age [d]/FCR×100. The specific study design followed for each individual trial is given in Table 2. Birds were distributed among 48 pens in 2 Petersime battery brooder units. Number of dietary treatments, total number of birds, and age at which birds were challenged are also illustrated in Table 2. The challenge model used to induce necrotic enteritis (**NE**) was adapted from [9].

Vaccine Administration

A commercial IBD vaccine (Schering–Plough Animal Health, Millsboro, DE) was used as a suppressant of humoral immunity in all studies, with the exception of the first study, in which Cocci-Vac (Schering-Plough Animal Health, Millsboro, DE) was spray-applied to the feed. The IBD vaccine was given via ocular route at a level 10x the manufacturer's recommended dose compromise the humoral immune system.

C. perfringens Challenge

Field isolates of *C. perfringens* (Georgia and Texas combined cultures) known to cause NE were isolated, cultured separately, and then combined [9]. The isolates were grown in thioglycollate medium for 12 h, and fresh inoculum was administered each d. Each bird received the *C. perfringens* challenge (3 mL administered by oral gavage to the crop) on days as illustrated in Table 2.

Study 1 The 2 YCW additives evaluated in this study were Safmannan and a partially hydrolyzed Safmannan at the rate of 0 (Control), 125, 250, 375, and 500 ppm for a total of 9 dietary treatments. A total of 240 newly hatched broiler chicks were randomly distributed among 48 pens with 5 birds/pen, yielding 8 replicates for the control and 5 replicates for each YCW treatment.

Study 2 The YCW products used were Pronady (at 125 and 250 ppm), Biosaf (at 1,000 ppm), and a combination of Biosaf and Pronady (at 1,000+125 and 1,000+250, ppm respectively). There were 8 replicates for each of the 6 dietary treatments.

Study 3 Effects of dietary supplementation of 2 additives, Pronady and Safmannan, at a concentration of 0, 125, 250, 375, and 500 ppm were investigated in this study. There were 9 dietary treatments with 8 replicates for the Control and 5 replicates for each YCW treatment.

Study 4 Feed was supplemented with one of the 2 additives (Pronady at 125 and 250 ppm or Safmannan at 125, 250, and 500 ppm). Two additional treatments consisted of a blend of 80% Pronady with 20% Safmannan at a total final concentration of 125 or 250 ppm. There were a total of 8 dietary treatments with 6 replicates/treatment.

Study 5 The basal diet was supplemented with one of 2 additives (Pronady or Safmannan) at the rate of 0, 125, 250, or 500 ppm. One additional treatment consisted of a blend of 50% Pronady and 50% Safmannan at a total final concentration of 134 ppm. There were a total of 8 dietary treatments with 6 replicates/treatment. This study was terminated on d 20 (1 d earlier than all other studies) because of high mortality.

Study 6 This study investigated YCW products from various sources (different manufacturing plants) individually and in combination. All dietary treatments were evaluated at 250 ppm. The basal diet was supplemented with one of the 3 additives or a blend [BR Pronady (**BRP**) manufactured in Brazil; CR Safmannan (**CRS**) manufactured in Cedar Rapids, IA; and FR Safmannan (**FRS**) manufactured in France]. Dietary treatments evaluated were Control, BRP, CRS, FRS, equal blend of BRP+CRS, equal blend of BRP+FRS, equal blend of CRS+FRS, and equal blend of BRP+CRS+FRS. There were a total of 8 dietary treatments with 6 replicates/treatment.

Statistical Analysis

Data obtained from the 6 individual studies were pooled and analyzed for the main effects of source (Control, Pronady, Safmannan, and blend), dose (0, 125, 250, 375, and 500) and for the interaction of source and dose using the General Linear Model (GLM) procedure (multivariate analysis) by including experiment as a fixed factor. The Biosaf 1,000 ppm treatment from Study 2 was excluded from the data and the partially hydrolyzed Safmannan treatment from Study 1 was considered as a Safmannan source. Broilers receiving any combination treatment in all experiments were considered as a common "Blend" group. In all studies, data from broilers receiving any concentration of Safmannan, Pronady, and Blend were evaluated for the effect of source and data from birds receiving 0, 125, 250, 375, and 500 ppm of any YCW additive were analyzed for the effect of dose. Variables analyzed were BW, WG, FC, FCR, PI, and MORT. Significant means ($p \leq 0.05$) were separated using Duncan's multiple range test [10].

RESULTS AND DISCUSSION

Pooled data analysis from all studies revealed that including a YCW additive had significant beneficial effects on broiler performance (Table 3). No significant differences were detected between the two sources of YCW: Safmannan, the baker's yeast product; and Pronady, the brewer's yeast product. There was no significant interaction between source and dose. Safmannan and Pronady treatments performed significantly better compared to the Control and resulted in an overall improvement of 10% growth rate with no difference in the FCR. The Blend (mix of Pronady and Safmannan) produced

Table 3. Effects of source and dose of prebiotic YCW products on the performance of 21-day-old broilers (pooled data from 6 studies).

	n	BW (g)	WG (g)	FC (g)	FCR	PI	MORT (%)
				Main effects of source			
Control	42	756^{c}	713	1,149	1.52	199	16.0
Safmannan[1]	98	841^{b}	799	1,200	1.43	242	14.5
Pronady[2]	78	829^{b}	786	1,157	1.40	245	14.4
Blend[3]	58	877^{a}	832	1,212	1.38	261	14.2
PSEM[4]		10.3	10.3	17.9	0.01	7.0	2.1
				Main effects of dose			
0	42	756^{c}	713	1,149	1.52	199^{c}	16.0
125	71	844^{a,b}	801	1,177	1.40	259^{a}	13.4
250	107	860^{a}	816	1,206	1.41	247^{a,b}	15.3
375	19	842^{a,b}	802	1,197	1.42	223^{b,c}	16.8
500	37	809^{b}	767	1,154	1.43	240^{a,b}	12.2
PSEM[4]		13.0	13.0	22.5	0.02	8.8	2.7
				ANOVA			
Source		0.05	0.07	0.14	0.10	0.07	0.41
Dose		0.05	0.07	0.17	0.88	0.02	0.30
Source × dose		0.54	0.57	0.57	0.60	0.30	0.11

[1]YCW product derived from baker's yeast.

[2]YCW product derived from brewer's yeast.

[3]Blend of Safmannan and Pronady.

[4]PSEM = Pooled SEM.

[a–c]Means for main effects within a column with no common superscript differ ($P \leq 0.05$).

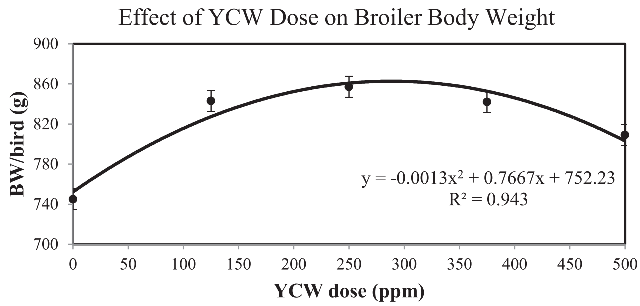

Figure 1. Quadratic effect of the dose of prebiotic YCW products on the body weight of 21-day-old broilers. The optimal dose of YCW needed to maximize this model was calculated to be 295 ppm.

better effects, with a significant improvement of about 15% in the BW and a 10% improvement in feed conversion when compared to the Control. All doses of YCW displayed significant improvement compared to the Control and produced higher BW and improvements in the PI. The dose effect of YCW on BW is demonstrated in Figure 1 (BW was fit to the YCW dose in a quadratic regression line; $R^2 = 0.943$), with the peak response of 865 g/bird calculated at a concentration of 295 ppm YCW. The 500 ppm dose had significantly lower BW when compared to 250 ppm. Supplementation of YCW product at 125 ppm produced a higher PI compared to all other doses. There were no significant differences observed with respect to mortality.

This study indicated that dietary inclusion of prebiotic YCW improves BW, WG, and PI, and reduces FCR in starter broilers that have been placed under an immune stress and a *C. perfringens* challenge. The improved BW in YCW supplemented broilers is likely due to the prebiotic functionality of YCW-derived MOS, which has been reported to promote the colonization of beneficial bacteria, improve intestinal integrity, and enhance immune functions [11–14]. In this study, no differences in production parameters were observed between the 2 sources of YCW products tested, however the blended product produced a higher BW with no difference in FCR compared to individual YCW products fed alone. This greater effect may be due to a "broader" immune response elicited by the mannoproteins present in each YCW product. The outer surface of YCW contains mannoproteins, which are covalently linked to β-glucans on the inner layer [15]. Even though the YCW products evaluated in this study were derived from the same species (*S. cerevisiae*), baker's yeast and brewer's yeast strains likely have different mannoproteins and stimulate a broader immune response when blended.

It has been reported that YCW products did not always produce consistently beneficial results on broiler production. Inconsistent results in the published literature may be due to different levels and sources of the products supplemented in the diets, and perhaps may be due to variability in the stress factors encountered by the birds. The current results were in accordance with some of the previous reports and contradictory to others. Bio-Mos supplemented at 3,000 ppm did not influence BW, FCR, or nutrient utilization [16] and in contrast, Bio-Mos at the same level significantly improved BW and FCR in finishing broilers [17]. Bio-Mos at 1,000 ppm in starter feed and 500 ppm in the grower and finisher feeds improved BW without changing the FCR [18]. Variation in the YCW composition was been reported to be based on the strain origin and the commercial process applied to concentrate the YCW product [19]. Therefore, the efficiency of YCW as a feed additive may differ depending on the source when it comes to improving broiler performance.

The current study revealed that the optimum level of prebiotic YCW in starter broiler diets exhibits a quadratic response that was maximal at approximately 295 ppm. It has been reported that diets supplemented with Safmannan at a rate of 500 ppm did not affect BW, FCR, or MORT in starter, grower, or finisher broilers under non-stress conditions [18]. The research available on the effects of prebiotics on *C. perfringens* infection in broilers is limited. *In vivo* studies in mammals and in vitro models revealed that fructo-ologosaccharides in diets resulted in significantly fewer *C. perfringens* bacteria in the intestinal tract [20, 21]. In the present study, supplementation of YCW in broiler diets resulted in improved performance under a simulated immune stress and *C. perfringens* challenge. Immunosuppressed chickens are more likely to develop NE. Successful challenge conditions will compromise the immune system and predispose the birds to NE [22]. YCW products act as immune-modulating substances, which may stimulate gut-associated and systemic immunity by acting as a nonpathogenic microbial antigen, giving an adjuvant-like effect [11]. This suggests a mechanism whereby the YCW blend may stimulate a broader immune response. Improved humoral immune responses have also been observed in birds fed YCW products [14, 23]. A meta-analysis conducted in 2004 revealed that broiler diets containing "MOS" improved final BW by 1.75% compared to negative control diets [24]. It is likely that the "MOS" described in the meta-analysis were not all pure mannanoligosaccharides, as it was fairly common in earlier literature to describe heterogeneous YCW products broadly as "MOS," which is not technically correct. In 2010, The Texas State Chemist banned the characterization of YCW additives as "MOS" since they are more precisely described as a mixture of a variety of compounds present in YCW.

Dietary supplementation of prebiotic YCW products produced growth improvement in starter broilers when under experimentally induced challenge conditions. The findings of the current study are commercially important as previous literature has suggested an optimal dose of 500 to 1000 ppm for YCW additives in starter broilers, whereas we have concluded that 250 to 300 ppm can maximize BW. Also, this is the first kind of investigation which evaluated different sources of YCW along with a blend of YCW products.

CONCLUSIONS AND APPLICATIONS

1. Diets supplemented with prebiotic YCW products resulted in improved BW with a decrease in FCR in starter broilers under experimentally induced challenge conditions.
2. Supplementation of a blended YCW product produced better growth performance in starter broilers compared to the individual products fed alone, and the individual products improved performance when compared to a control. The source of the YCW product had no effect on performance.
3. This study suggests that the optimal level of prebiotic YCW products as a growth promoting feed additive in starter broiler diets is approximately 250 to 300 ppm.

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