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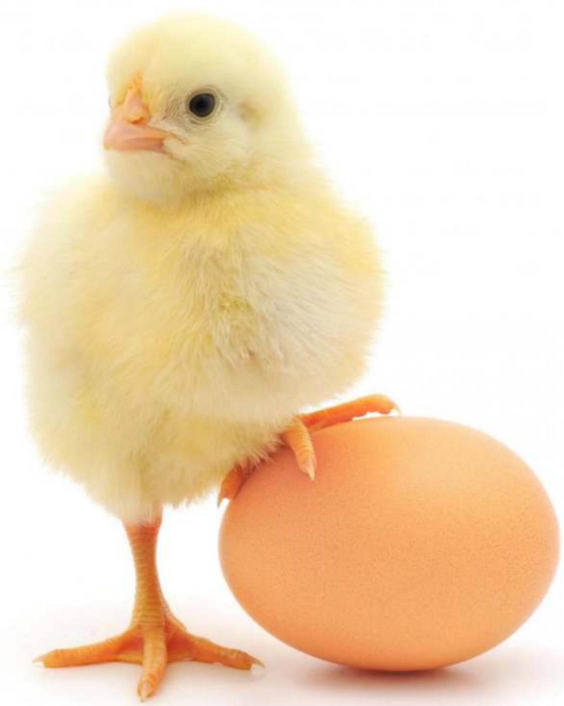

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Research Article
Combination of Xylanase and *Bacillus* Direct-fed Microbials, as an Alternative to Antibiotic Growth Promoters, Improves Live Performance and Gut Health in Subclinical Challenged Broilers

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Abstract
Objective: This study evaluated the effect of xylanase, *Bacillus* direct-fed microbials (DFM) and their combination on performance under a mild, subclinical challenge with two *Eimeria* species and *Clostridium perfringens* in broilers raised to 42 days. **Materials and Methods:** A total of 6 dietary treatments were used throughout the trial. Diets were supplemented with one of the following; no xylanase or *Bacillus* (control), xylanase only, *Bacillus* L. only, *Bacillus* A. only, xylanase plus *Bacillus* L. or xylanase plus *Bacillus* A. Data were analyzed as randomized complete block design. **Results:** When compared to control at 42 days, the xylanase, *Bacillus* L. and *Bacillus* A. improved ($p \leq 0.05$) BW gain by 93, 94 and 53 g, respectively and FCR by 4, 4 and 6 points, respectively. When compared to control at 42 days, the combination of xylanase and *Bacillus* L. or *Bacillus* A. improved ($p \leq 0.05$) BW gain by 142 or 147 g, respectively and FCR by 9 or 11 points, respectively. The combination of xylanase and *Bacillus* L. or *Bacillus* A. reduced ($p \leq 0.05$) BW coefficient of variation from 15.09% (control) to 8.27 or 8.22%, respectively at 42 days. The combination of xylanase and *Bacillus* L. or *Bacillus* A. reduced ($p \leq 0.05$) gross lesion scores in small intestine and *C. perfringens* count at 42 days compared to control. **Conclusion:** Results suggest that xylanase and *Bacillus* alone may improve broiler performance and reduce the severity of intestinal lesions due to *Eimeria* and *C. perfringens* challenges and that the effect of xylanase and *Bacillus* DFM are additive.

Key words: Broiler, xylanase, direct-fed microbial, *Clostridium perfringens*, *Eimeria*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sub-therapeutic levels of antibiotics have been used in feed as growth promoters (AGP) in poultry and other species in an attempt to control enteric diseases and improve feed conversion since the 1940s[1]. However, the indiscriminate use of AGPs in production animals has resulted in the emergence of antibiotic-resistant pathogens, which has serious human health implications[2]. With the recent FDA guidance to voluntarily reduce antibiotics for the use of growth promotion, the demand for alternative solutions has risen. This has led to increased interest in the use of feed additives in feed formulation to promote growth and limit pathogen presence.

One potential AGP alternative is the use of exogenous enzymes, which are commonly used to improve the nutritive value of feedstuffs. Among these are carbohydrases, their mode of action is predominantly through degradation of non-starch polysaccharides (NSP), which are the major dietary fiber component within the cell wall of cereal grains[3]. Specifically, xylanases can improve the nutritive value by either reducing digesta viscosity[4], releasing encapsulated nutrients by disruption of the cell wall structure[5] or through a prebiotic effect in which xylo-oligosaccharides (XOS) are produced by xylanase. The XOS can be utilized as a substrate for beneficial bacteria thus help in improving intestinal health[6], consequently, improving poultry performance[7].

Another option for AGP alternatives are direct-fed microbials (DFM) or probiotics. The DFM help positively shift the balance of the microbial population in the gastro-intestinal tract (GIT), improve digestion and absorption of nutrients and to inhibit the growth of pathogenic microorganisms[8]. A wide range of microorganisms have been used as DFM in commercial poultry production, with *Bacillus* and *Lactobacillus* constituting the majority of species used[8]. It has been shown that *Bacillus subtilis* DFM can reduce clinical signs of experimental avian coccidiosis with concomitant increase in protective immunity[9] and improve growth performance of chickens[10]. The DFM are typically naturally occurring microorganisms supplemented as either dried or soluble fermentation products.

The interaction between exogenous enzymes and DFMs has been reported in the literature[11-13]. It is well known that feed enzymes, such as carbohydrases, can improve the utilization of carbohydrates and provide "prebiotics" to beneficial bacteria, whereas proteases can reduce the indigestible protein and reduce substrate availability for pathogenic bacteria. The DFMs can improve the microbial balance, intestinal health and provide an environment that may stimulate the activity of enzymes[8].

The objective of this study was to investigate the impact of combining *Bacillus* DFMs and xylanase enzyme on improving the live performance and gut health of broilers challenged with *Clostridium perfringens* and multiple *Eimeria* spp.

MATERIALS AND METHODS

Experimental design: A total of 2,496 Ross 708 mixed sex 1-day old broilers (males and females) were obtained from a commercial hatchery and placed in floor pens to evaluate the effect of xylanase, DFMs and the combinations of xylanase and DFMs on live performance, coccidiosis observations (severity of intestinal lesion scores), *Salmonella* incidence, *C. perfringens*, *Escherichia coli* and aerobic plate count (APC) intestinal load. The experiment was conducted in a completely randomized block design and consisted of 6 experimental treatments with 8 replicates per treatment, each containing 52 chicks, for a 42 days assay period.

Chicks were reared on used litter in 5×10 ft. floor pens with a minimum of 0.87 ft^2 per bird, provided age appropriate environmental conditions and given access of feed and water *ad libitum*. The lighting program included continuous light for the first week (>3 fc), then dimmed to 1 fc for the remainder of the trial. Litter was dosed with *C. perfringens*, *E. maxima* and *E. acervulina* at day 1, 7 and 10 post-hatch (Table 1). Mortality was recorded as it occurred and a record of mortality weight was kept. On day 21, 35 and 42, at each dietary change, birds and remaining feed were weighed for determination of average body weight (BW), feed consumption for the calculation of mortality-adjusted feed conversion ratio (FCR) and BW coefficient of variation (flock uniformity). Intestinal lesions, as indication of coccidiosis, were scored on day 21 and 42 of age (2-males and 2-females per pen at each age). *Clostridium perfringens* count per gram of intestinal content, *E. coli* count and *Salmonella* incidence were detected at 21 and 42 days of age.

Animal care practices conformed to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching[14].

Table 1: Bacterial dose of challenge and the timeline of administration, post hatch

Species	Bacterial dose (cells per bird)	Day of challenge (post hatch)
C. perfringens	$>10^7$	1
E. acervulina	10^4	7
E. maxima	10^3	10

Table 2: Ingredients, calculated nutrients concentration of the control diet for the starter (day 0-21), grower (day 22-35) and finisher (day 36-42) dietary phases

Ingredients (%)	Starter	Grower	Finisher
Corn	57.51	64.44	67.77
Soybean meal 48%	27.44	22.28	19.92
Soy oil	1.00	1.00	1.00
Poultry by-product meal	5.00	5.00	5.00
DL-methionine	0.20	0.15	0.11
Salt	0.43	0.43	0.43
L-Lysine HCL	0.15	0.19	0.11
Limestone	5.40	3.92	3.23
Di-calcium phosphate	2.22	1.96	1.80
Choline chloride 60%	0.03	0.00	0.00
Minerals and vitamins premix[1]	0.63	0.63	0.63
Calculated nutrient concentration			
Crude protein (%)	22.00	20.00	19.00
Crude fat (%)	4.35	4.49	4.56
Calcium (%)	1.05	0.90	0.85
Available phosphate (%)	0.50	0.45	0.42
Dig lysine (%)	1.28	1.15	1.02
Dig TSAA (%)	0.93	0.86	0.80
Dig tryptophan (%)	0.12	0.15	0.12
Dig threonine (%)	0.82	0.70	0.60
Dig arginine (%)	1.30	1.20	1.00
Sodium (%)	0.20	0.20	0.20
Metabolizable energy (kcal kg^{-1})	2930.00	3029.00	3080.00

[1]Vitamin premix supplied the following per kg of diet: 5,512 IU vitamin A, 1,852 IU vitamin D3, 11 IU vitamin E, 0.06 mg vitamin B12, 0.23 mg biotin, 1.87 mg menadione (K_3), 0.44 mg thiamine, 3.75 mg riboflavin, 5.95 mg d-pantothenic acid, 1.32 mg vitamin B6, 34.17 mg niacin and 0.22 mg folic acid. The Mineral premix supplied the following per kg of diet: Manganese: 120 mg, zinc: 120 mg, Iron: 80 mg, Copper: 10 mg, Iodine, 2.5 mg, Cobalt, 1 mg

Experimental diets: Diets were formulated to either meet or exceed the NRC[15] nutrient requirements for broilers and provided in three phases: Starter (day 0-21), grower (day 22-35) and finisher (day 36-42). The 6 dietary treatments consisted of an unsupplemented negative control (no DFM and no xylanase, NC), NC+*Bacillus* L., NC+*Bacillus* A., NC+xylanase, NC+*Bacillus* L.+xylanase and NC+*Bacillus* A.+xylanase. The xylanase is an endo-β-1, 4-xylanase derived from *Orpinomyces* species and produced by *Pichia pastoris* strain added at an inclusion level of 15 XU/g of feed. Two DFMs, *Bacillus* L. and *Bacillus* A., were used in this study at an inclusion level of 1×10^6 CFU/g of feed. All diets were corn-soybean meal-based (Table 2). Inclusion levels were consistent at all phases.

Statistical methods: A one-way ANOVA using completely randomized design with 8 replicate pens per dietary treatment was employed. The GLM of SAS[16] was used to analyze the data and differences among means were partitioned by LSMEANS. The experimental unit for live performance parameters was the pen, while for microbial and lesion score data the experimental unit was an individual bird.

RESULTS AND DISCUSSION

Live performance: Throughout the entire study (1-42 days of age) inclusion of either *Bacillus* L. and *Bacillus* A. alone, xylanase alone or a combination of *Bacillus* L. plus xylanase or *Bacillus* A. plus xylanase, significantly ($p<0.05$) improved 21 and 42 days broiler performance as compared to the negative control (Table 3).

The BW and FCR were improved ($p<0.05$) by the combination of either *Bacillus* L. or *Bacillus* A. with xylanase (Table 3). The combination of either *Bacillus* A. or *Bacillus* L. with xylanase improved FCR at 42 days compared to single product supplementation. Zhang and Kim[17] reported that when multi-strains of *Bacillus* are fed to broilers to 35 days improved body weight gain (BWG), FCR, but not feed intake is observed, as found in this study. Treatment of pen litter with *C. perfringens*, *E. maxima* and *E. acervulina* resulted in a mortality of 4.79% at 42 days. Supplementation of *Bacillus* A., *Bacillus* L. or xylanase alone resulted in reduce ($p<0.5$) mortality. This is in agreement with Lee *et al*.[9], who reported that DFMs have shown promise as an alternative to in-feed antibiotics in reducing enteric disease and eliminating subsequent contamination of poultry products. The combination of enzyme and either DFM reduced mortality ($p<0.05$) compared to negative control, suggesting an additional benefit when xylanase is utilized in combination with DFMs.

Intestinal pathogen load: Results for lesion score, *E. coli*, APC and *C. perfringens* count and *Salmonella* incidence (%), measured at 21 and 42 days of age are presented in Table 4. Supplementing diets with the combination of *Bacillus* L. or *Bacillus* A with xylanase were found to improve ($p<0.05$) lesion scores at 14, 21 and 42 days and reduce ($p<0.05$) intestinal bacteria counts of APC and *E. coli* counts and *Salmonella* incidence (Table 4). This is likely due to the DFM functioning to maintain the presence of beneficial microorganisms and inhibit growth of pathogenic bacteria.

Single product supplementation significantly reduced ($p<0.05$) the levels of *C. perfringens* in small intestines (1.22 CFU/g xylanase, 0.80 CFU/g *Bacillus* A. and 0.82 CFU/g *Bacillus* L. log reduction). All combinational supplementation outperformed single product supplementation. A log reduction of 1.49 CFU/g in *C. perfringens* with the combination of xylanase and *Bacillus* A. and 1.55 with the combination of xylanase and *Bacillus* L. was observed. Similar results are observed when measuring the *Salmonella* incidence in intestinal contents across treatments. Single product supplementation significantly reduced ($p<0.05$)

Table 3: Xylanase alone or probiotics and the combination of xylanase and probiotics effects on broiler body weight (BW), body weight gain (BWG), feed intake (FI) and mortality-adjusted feed conversion ratio (FCR)

	Treatments							
Variable/age	Negative control	*Bacillus* L.	*Bacillus* A.	Xyl	*Bacillus* L.+Xyl	*Bacillus* A.+Xyl	SEM[1]	p-value
BW (g)								
21	832.00^{c}	856.00^{ab}	851.00^{b}	859.00^{ab}	871.00^{a}	871.00^{a}	2.88	<0.0001
35	1954.00^{c}	2020.00^{ab}	1990.00^{bc}	2028.00^{ab}	2054.00^{a}	2048.00^{a}	7.31	<0.0001
42	2746.00^{c}	2841.00^{ab}	2800.00^{bc}	2840.00^{ab}	2889.00^{a}	2894.00^{a}	10.49	<0.0001
BWG (g)								
1-21	784.00^{c}	808.00^{ab}	802.00^{b}	811.00^{ab}	823.00^{a}	822.00^{a}	2.86	<0.0001
22-35	1122.00^{c}	1164.00^{ab}	1139.00^{bc}	1169.00^{ab}	1182.00^{a}	1177.00^{a}	5.99	<0.02
36-42	792.00	821.00	810.00	811.00	835.00	846.00	8.88	0.59
FCR (g:g)								
1-21	1.36^{a}	1.33^{b}	1.31^{c}	1.34^{b}	1.30^{c}	1.31^{c}	0.003	<0.0001
1-35	1.64^{a}	1.61^{b}	1.58^{c}	1.61^{b}	1.57^{cd}	1.56^{d}	0.005	<0.0001
1-42	1.90^{a}	1.86^{bc}	1.84^{c}	1.86^{b}	1.81^{d}	1.79^{d}	0.006	<0.0001
Mortality (%)								
1-21	2.94^{a}	1.47^{ab}	0.98^{b}	0.98^{b}	0.74^{b}	0.49^{b}	0.24	<0.04
1-35	4.26^{a}	1.86^{b}	1.33^{b}	1.33^{b}	0.80^{b}	0.80^{b}	0.30	<0.004
1-42	4.79^{a}	2.13^{b}	1.33^{b}	1.33^{b}	1.06^{b}	0.80^{b}	0.34	<0.003
Flock uniformity (%)								
1-21	15.03^{a}	10.81^{b}	10.79^{b}	10.77^{b}	8.23^{c}	8.63^{c}	0.34	<0.0001
1-42	15.09^{a}	11.03^{b}	10.89^{b}	10.80^{b}	8.27^{c}	8.23^{c}	0.39	<0.0001

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($p \leq 0.05$). [1]Standard error of the mean (SEM) for n = 8 floor pens of 52 birds/pen

Table 4: Xylanase alone or probiotics and the combination of xylanase and probiotics effects on lesion scores, *E. coli*, aerobic plate count (APC), Salmonella incidence and *Clostridium* perfringens

	Treatments							
Variable/age	Negative control	*Bacillus* L.	*Bacillus* A.	Xyl	*Bacillus* L.+Xyl	*Bacillus* A.+Xyl	SEM[1]	p-value
Lesion score								
14	1.63^{a}	1.00^{b}	0.63^{bc}	0.50^{c}	0.25^{c}	0.13^{c}	0.10	<0.0001
21	1.28^{a}	1.03^{ab}	0.75^{c}	0.94^{bc}	0.38^{d}	0.28^{d}	0.06	<0.0001
42	1.21^{a}	1.06^{ab}	0.93^{b}	1.03^{ab}	0.43^{c}	0.26^{c}	0.06	<0.0001
E. coli ($\log_{10}$ CFU/mL)								
21	7.02^{a}	6.43^{ab}	6.05^{bc}	6.30^{b}	5.89^{bc}	5.55^{c}	0.11	<0.0001
42	7.27^{a}	6.29^{b}	5.80^{cd}	6.20^{bc}	5.58^{d}	5.57^{d}	0.11	<0.0001
APC ($\log_{10}$ CFU/mL)								
21	9.06^{a}	7.23^{bc}	7.54^{b}	7.17^{bc}	6.98^{bc}	6.80^{c}	0.14	<0.0001
42	8.95^{a}	7.27^{b}	7.03^{bc}	7.17^{bc}	6.84^{c}	6.89^{c}	0.12	<0.0001
Salmonella Incidence (%)								
21	71.90^{a}	43.80^{b}	37.50^{bc}	31.30^{bcd}	18.80^{cd}	12.50^{d}	3.86	<0.0001
42	70.00^{a}	36.60^{bc}	31.30^{bcd}	40.00^{b}	21.30^{cd}	20.00^{d}	3.21	<0.0001
C. perfringens ($\log_{10}$ CFU/g)								
21	4.02^{a}	3.29^{b}	2.87^{c}	3.05^{bc}	2.26^{d}	2.22^{d}	0.11	<0.0001
42	4.06^{a}	3.24^{b}	3.26^{b}	2.84^{c}	2.51^{cd}	2.57^{d}	0.09	<0.0001

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($p \leq 0.05$), [1]Standard error of the mean (SEM) for n=8 floor pens of 32 birds/pen

Salmonella incidence (30% xylanase, 39% *Bacillus* A., 33% *Bacillus* L.). The combinational supplementation outperformed single product supplementation in reducing *Salmonella* incidence, with a 50 and 48.8% decrease for the combination of xylanase with *Bacillus* A. and *Bacillus* L., respectively. The combination of DFM and xylanase likely have a combinatorial effect on improving the survival of beneficial microorganisms and their colonization in the intestinal tract.

CONCLUSION AND FUTURE RECOMMENDATIONS

It can be concluded that supplementing xylanase to broiler feed may improve live performance and gut health. Furthermore, supplementing *Bacillus* DFMs can also improve live performance and reduce pathogen load in the digestive tract when supplemented to broilers up to 42 days. When xylanase and *Bacillus* DFM supplemented simultaneously, FCR and lesion score were improved further (additive effect).

SIGNIFICANCE STATEMENT

Current study shows that xylanase and Bacillus DFM can be used in combination to improve live performance and reduce pathogen load in an antibiotic free environment. These findings are significant given the increased interest in AGP-free poultry production.

REFERENCES

1. Dibner, J.J. and J.D. Richards, 2005. Antibiotic growth promoters in agriculture: History and mode of action. Poult. Sci., 84: 634-643.
2. Lekshmi, M., P. Ammini, S. Kumar and M.F. Varela, 2017. The food production environment and the development of antimicrobial resistance in human pathogens of animal origin. Microorganisms, Vol. 5, No. 1. 10.3390/microorganisms5010011.
3. Slominski, B.A., 2011. Recent advances in research on enzymes for poultry diets. Poult. Sci., 90: 2013-2023.
4. Bedford, M.R. and H.L. Classen, 1992. Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. J. Nutr., 122: 560-569.
5. Meng, X., B.A. Slominski, C.M. Nyachoti, L.D. Campbell and W. Guenter, 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. Poult. Sci., 84: 37-47.
6. Bedford, M.R. and A.J. Cowieson, 2012. Exogenous enzymes and their effects on intestinal microbiology. Anim. Feed Sci. Technol., 173: 76-85.
7. Courtin, C.M., W.F. Broekaert, K. Swennen, O. Lescroart and O. Onagbesan *et al*., 2008. Dietary inclusion of wheat bran arabinoxylooligosaccharides induces beneficial nutritional effects in chickens. Cereal Chem., 85: 607-613.
8. Seo, J.K., S.W. Kim, M.H. Kim, S.D. Upadhaya, D.K. Kam and J.K. Ha, 2010. Direct-fed microbials for ruminant animals. Asian-Aust. J. Anim. Sci., 23: 1657-1667.
9. Lee, K.W., H.S. Lillehoj, S.I. Jang, G.X. Li, S.H. Lee, E.P. Lillehoj and G.R. Siragusa, 2010. Effect of *Bacillus*-based direct-fed microbials on *Eimeria maxima* infection in broiler chickens. Compa. Immunol. Microbiol. Infect. Dis., 33: e105-e110.
10. Deniz, G., A. Orman, F. Cetinkaya, H. Gencoglu, Y. Meral and I.I. Turkmen, 2011. Effects of probiotic (*Bacillus subtilis* DSM 17299) supplementation on the caecal microflora and performance in broiler chickens. Rev. Med. Vet., 162: 538-545.
11. Walsh, M.C., L.F. Romero, S.E. Indrakumar and V. Ravindran, 2013. Influence of combinations of a direct-fed microbial and exogenous enzymes on the growth performance and feed efficiency of broilers. Poult. Sci., 92(E-Suppl. 1): 87-87.
12. Murugesan, G.R. and M.E. Persia, 2013. Effects of exogenous enzymes and direct-fed microbials on performance, energy utilization and body composition of broiler chicks. Poult. Sci., 92(E-Suppl. 1): 10-10.
13. Murugesan, G.R., I.V. Wesley, J. Remus, P.W. Plumstead and M.E. Persia, 2013. Effects of exogenous enzymes and direct-fed microbials supplementation on first-cycle laying hen performance, energy digestibility, gut integrity and pathogen colonization. Poult. Sci., 92(E-Suppl. 1): 11-11.
14. FASS., 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd Edn., Federation of Animal Science Societies, Champaign, IL., USA.
15. NRC., 1994. Nutrient Requirements of Poultry. 9th Rev. Edn., National Academy Press, Washington, DC., USA., ISBN-13: 978-0309048927, Pages: 176.
16. SAS., 2011. The SAS System for Windows 7. Release 9.3. SAS Institute Inc., Cary, NC.
17. Zhang, Z.F. and I.H. Kim, 2014. Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding and excreta odor contents in broilers. Poult. Sci., 93: 364-370.