



EVALUATION OF PROBIOTIC, PREBIOTIC AND SYNBIOTIC ON STARTER BROILERS PERFORMANCE SUBJECTED TO BURSA VACCINE AND *CLOSTRIDIUM PERFRINGENS* CHALLENGE

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ABSTRACT

The effect of commercial alternative to antibiotic individual or combination has been used in starter broiler diet on broiler performance. A total of 288 straight run broiler chicks Ross 308 were allocated within 2 Petersime battery units. A total of 7 treatments were distributed randomly in completely randomize design to 48 pens by 6 birds/ pen. Each treatment had 7 replicates pens except non-challenge control had 6 replicates pens. Treatments 1 and 2 were non-challenged (NCh-C) and challenged (Ch-C) without any additives. Treatment 3 basal diet plus prebiotic (Yeast Cell Wall) 250 ppm (YCW). While, treatment 4 fed basal diet plus probiotic (*Bacillus subtilis* 3×10^5 cfu/g feed) 300 ppm (Pro300). Treatment 5 fed basal diet with probiotic (*Bacillus subtilis* 1×10^5 cfu/ g feed) 100 ppm (Pro100). Treatment 6 fed basal diet with combination YCW (250 ppm) plus Pro300 (300 ppm) (YCW+Pro300). Treatment 7 fed basal diet with combination of YCW (250 ppm) plus Pro100 (100 ppm) (YCW+Pro100). All birds were vaccinated commercial Infectious Bursal Disease IBD vaccine by eye drop at day 10 of age then followed by 3 ml oral gavages *Clostridium Perfringens* challenge 10^6 cfu/ml on days 16, and 17. There is no significant difference (P 0.05) among treated groups for all performance traits. In contrast, adding of (YCW), (Pro100) then (Pro300) respectively were improved numerically, but not significantly (P 0.05) feed conversion ratio (FCR), body weight (BW), body weight gain (BW gain), and productivity index PI on day 10, 16, and 21. In conclusion, feeding adjusting the nutrient of our diet to be similar to Ross 308 guide led to enhance of performance by adding probiotic and prebiotic but not significantly. Probiotic and prebiotic did not appear their effect positively may be due to chicks reached to maximum BW by the effect of high density ration.

KEYWORDS: Broiler, performance, prebiotic, probiotic.

INTRODUCTION

Intensive research to find natural growth promoters to better health and protection against pathogens especially after European Union 2006 banned antibiotic growth promoters AGPs usage (Ganan *et al.*, 2012); Huyghebaert *et al.* (2011). Also, United State initiated to remove AGPs from feed by Food and Drug Administration 2013. Huyghebaert *et al.* (2011) found that removal of AGPs from poultry diet as antimicrobial led to poor performance and appear subclinical necrotic enteritis beside of dysbacteriosis. Poultry industry and feed additive specialist are looking for to find alternative product such as probiotic, prebiotic, synbiotic, plant extract and essential oil. All this product may enhance immunity and growth performance (Al-Kassie *et al.*, 2009; Al-Kassie and Jameel, 2009; Al-Kassie *et al.*, 2008b; Jameel, 2008; Jameel *et al.*, 2014; Loh *et al.*, 2014; Sugiharto, 2016). In feed additive strategies, two goals have been obtained from adding prebiotic to broiler diet. Replacing AGPs to get same effect on enhancing performance, and reducing pathogenic bacteria and diseases by increasing beneficial bacteria in gastro intestinal tract GIT besides enhance productive traits (Callaway *et al.*, 2008; Gaggia *et al.*, 2010; Ricke, 2015). Prebiotic fermentation occurs in chicken's ceca. However, the major site of habituating zoonotic pathogens *Campylobacter* and *Salmonella* in ceca too (Hofacre *et al.*, 2005; Hume, 2011; Ricke, 2015).

Prebiotic in a non-digestible carbohydrate capable to influence selectively on intestinal bacteria and possible effect on bird immunity (Bozkurt *et al.*, 2014; Kim *et al.*, 2011). Prebiotic Yeast Cell Wall (YCW) contain mannan-oligosaccharide (MOS) and β -glucan (Lipke and Ovalle, 1998). It has been reported that (MOS) prevent gram negative bacterial infection by competitive exclusion in the GIT of chicken (Baurhoo *et al.*, 2007). Also, (MOS) may enhance immunity and intestinal mucosa ((Baurhoo *et al.*, 2007; Pourabedin *et al.*, 2014). Another ingredient of prebiotic is β -glucan, it has been reported the beneficial effect by improving innate immunity (non-specific) and growth performance (Chae *et al.*, 2006; Refstie *et al.*, 2010). Probiotic is a live non-pathogenic microbial feed additive that maintain microbial balance in GIT (Fuller, 1989). In poultry industry, the most common probiotic is *Bacillus subtilis* spores due to heat resistance during pelleting formation. Administration of *Bacillus subtilis* spores to chicken feed has been reported to lowering *Escherichia coli* (Teo and Tan, 2007), *Salmonella* (Park and Kim, 2014), in addition, improve BW gain and FCR (Fritts *et al.*, 2000). Mountzouris (2014) have been shown that adding probiotic to chicks diet led to improve performance. Also, maintenance and establishment of intestinal microbiota beneficially that may enhance beneficial colonization in the GIT against pathogens. Thus, the objective of this current study was to evaluate

the influence of adding probiotic, prebiotic and synbiotic on Starter Broilers Performance Subjected to Bursa Vaccine and *Clostridium perfringens* challenge on broiler performance.

MATERIALS & METHODS

Birds, Housing and Feeding

This study was carried out Texas A & M University College Station, Texas at Poultry Research Center, U.S. Department of Agriculture (USDA), from 9 Feb. 2016 to 1 Mar. 2016. A total of 288 straight run broiler chicks Ross 308 were allocated within 2 Petersime battery units. A total of 7 treatments were distributed randomly in completely randomize design to 48 pens by 6 birds/ pen. Each treatment had 7 replicates pens except non-challenge control had 6 replicates pens. Treatments 1 and 2 were non-challenged (NCh-C) and challenged (Ch-C) without any additives. Treatment 3 basal diet plus prebiotic (Yeast Cell Wall) 250 ppm (YCW). While, treatment 4 fed basal diet plus probiotic (*Bacillus subtilis* 3×10^5 cfu/g feed) 300 ppm (Pro300). Treatment 5 fed basal diet with probiotic (*Bacillus subtilis* 1×10^5 cfu/ g feed) 100 ppm (Pro100). Treatment 6 fed basal diet with combination YCW (250 ppm) plus Pro300 (300 ppm) (YCW+Pro300). Treatment 7 fed basal diet with combination of YCW (250 ppm) plus Pro100 (100 ppm) (YCW+Pro100). Basal broiler starter diet was prepared as starter pellets and was divided into 6

treatment groups equally, Feed and water provided *ad libitum* to the end of the study (day 21). All diets met the Aviagen broilers Ross 308 requirement table (1) showed starter feed formulation.

Disease control and Challenge:

There is no drug therapy was used. On the day of hatch, vaccines were administered at the standard Sanderson Farms Hatchery. Commercial IBD vaccine via eye drop was vaccinated all birds at 10 days of age. After that, birds received 3 ml oral gavage *Clostridium Perfringens* challenge 10^6 cfu/ml on days 16, and 17 except NCh-C group to induce immunosuppression and challenge.

Growth performance and Samples

Daily observation of general flock condition, water, ration, temperature, unexpected events for the house, and mortality for each pen. All birds were weighted and feed intakes were recorded at days 1, 10, 16, and 21 of the study. To get BW gain, calculation of initial BW from the final BW. The residual feed from the offered feed to get feed consumption. Data of BW gain and feed consumption were used to get FCR.

Statistical analysis:

Data were analyzed as one-way ANOVA using the general linear model (GLM) procedure of SPSS™ 22.0 software (Corp, 2011). Seven Treatment means were separated using a “protected” Duncan’s analysis.

TABLE 1: Feed composition of starter diets.

Ingredients	Composition
TAMU corn #21	62.24
TAMU Soybean	31.71
DL-Methionine 98	0.272
Lysine HCL	0.182
L-threonine 98	0.032
AV Blend 8500	1.99
Limestone	1.307
BIOFOS 16/21P	1.55
Salt	0.409
TAMU Trace. Mineral	0.050
TAMU Vitamins	0.250
Calculation composition	
Crude protein CP%	22.0
ME poultry kcal/kg	3000
Crude fat	3.5
Crude fiber	2.14
Calcium	0.9
AV Phosphate	0.45
AV-methionine	0.57
AV-Lysine	1.18
AV-Arginine	1.32
AV-TSAA	0.85
Threonine	0.73
Sodium	0.18
Potassium	0.92
Chloride	0.32

RESULTS & DISCUSSIONS

There are no significant differences (P 0.05) among treated groups for all performance traits. In contrast, adding of (YCW), (Pro100) then (Pro300) respectively

were improved numerically, but not significantly (P 0.05) feed conversion ratio (FCR), body weight (BW), body weight gain (BW gain), and productivity index PI on day 10, 16, and 21. Table (2) showed results of the experiment.

TABLE 2: Effect of Probiotic, Prebiotic, and Synbiotic on challenge phase BW, BW gain, PI, and FCR at day 10, 16, 21.

Days	Treatments						
	1	2	3	4	5	6	7
	NCH-C	CH-C	YCW	Pro300	Pro100	Pro300+YCW	Pro100+YCW
Body weight BW (g)							
10 d	298±12	300±12	304±21	302±16	308±16	307±18	299±9
16 d	588±24	595±21	604±30	595±31	611±27	588±68	599±23
21d	945±46	918±67	949±70	938±68	941±76	913±110	926±54
BW gain (gm)							
10 d	254±11	257±12	260±20	257±16	263±16	263±18	255±9
16 d	290±13	295±12	300±18	293±16	303±18	280±62	300±19
21d	358±28	323±54	345±45	343±41	330±60	325±68	326±36
Productivity Index PI							
10 d	253±13	252±17	258±16	259±18	263±21	250±17	246±12
16 d	285±12	284±16	294±14	291±18	299±17	268±38	267±31
21d	340±19	318±34	297±44	319±47	301±62	290±31	292±40
FCR							
10 d	1.00±0.01	0.99±0.02	1.00±0.04	1.00±0.02	1.00±0.03	1.02±0.03	1.01±0.02
16 d	1.19±0.01	1.18±0.02	1.19±0.05	1.18±0.02	1.19±0.02	1.23±0.07	1.20±0.02
21d	1.26±0.01	1.28±0.04	1.31±0.03	1.27±0.03	1.29±0.04	1.31±0.05	1.30±0.04

Many researchers have investigated the effect of prebiotic and probiotic on broiler growth performance (Abdaljaleel *et al.*, 2016; Al-Kassie *et al.*, 2008a; Al-Kassie *et al.*, 2009; Hajati and Rezaei, 2010; Hashim *et al.*, 2016; Mookiah *et al.*, 2014; Zhao *et al.*, 2013; Zhao *et al.*, 2016). Steiner (2006) who reported that this additive create beneficial condition in the intestine. It has been reported that prebiotic can stimulate microflora in the intestine (Calik and Ergün, 2015; Schumann, 2002). Dizaji *et al.* (2012) showed that supplemented broiler diet with 0.10% manna oligosaccharide led to increase BW and decrease FCR at days 15, 28, 29, and 42 as compared with birds fed basal diet. Santin *et al.* (2001) reported that use of prebiotic led to improve protein and energy utilization by increase length of intestine and area of absorption. Supplementation broiler diet with oligosaccharide increased nutrient digestibility of broilers and improve gut health (Tuohy *et al.*, 2003). (Patterson and Burkholder, 2003) showed that supplemented broiler diet with prebiotic led to improve gastrointestinal G.I. tract health. Adding of YCW led to improve growth performance of broilers; on the other hand, they have been investigated that adding of YCW did not affect on broiler performance (Cox *et al.*, 2010; Munyaka *et al.*, 2012). It has been reported that YCW product improve BW, BW gain, and FCR. Also, enhance beneficial microflora of G.I tract and gut development (Yang *et al.*, 2007). Yeast product increase goblet cell and villi height (Baurhoo *et al.*, 2007) and modulation of nonspecific immune response of broilers (Alizadeh *et al.*, 2016). Our results are agreement with (Yalçinkaya *et al.*, 2008) who reported that no significant differences after supplemented diet with prebiotic on BW gain. While Kim *et al.* (2011) observed that supplemented diet with prebiotic had a significant differences by increasing BW gain.

CONCLUSION

In conclusion, feeding adjusting the nutrient of our diet to be similar to Ross 308 guide led to enhance of performance by adding probiotic and prebiotic but not

significantly. Probiotic and prebiotic did not appear their effect positively may be due to chicks reached to maximum BW by the effect of high density ration.

REFERENCES

- Abdaljaleel, R., Al-Ajeeli, M., H., Mohammed, Jameel, Y., Alsadwi, A., and Bailey, C. (2016) Effect of yeast cell wall supplementation on threonine requirements in broilers as measured by performance and intestinal morphology, In: Poultry Science Association 105th Annual Meeting Abstracts, p. 46.
- Al-Kassie, G., Al-Jumaa, Y., Jameel, Y. (2008a) Effect of probiotic (*Aspergillus niger*) and prebiotic (*Taraxacum officinale*) on blood picture and biochemical properties of broiler chicks. International Journal of Poultry Science 7, 1182-1184.
- Al-Kassie, G., Al-Jumaa, Y., Jameel Y. (2009) A Comparison Study of Adding Probiotic (*Aspergillus niger*) and Prebiotic (*Taraxacum officinale*) in Diet and its Effect on Performance of Broiler Chicks. Life Science International Journal 3, 948-951.
- Al-Kassie, G., Jameel, Y. (2009) The effect of adding *Thyme vulgaris* and *Cinnamomum zeylanicum* on productive performance in broilers, In: Proceeding of 9th Veterinary Scientific Conference, College Vet. Med., Univ. Baghdad, Iraq.
- Al-Kassie, G., Mohammed, M., Hamood, M., Jameel, Y.J. (2008b) The effect of anise and rosemary on the microbial balance in gastro intestinal tract for broiler chicks. International Journal of Poultry Science 7, 610-612.
- Alizadeh, M., Rodriguez-Lecompte, J.C., Yitbarek, A., Sharif, S., Crow, G., Slominski, B.A. (2016) Effect of yeast-derived products on systemic innate immune response of broiler chickens following a lipopoly saccharide challenge. Poultry science 95, 2266-2273.

- Baurhoo, B., Letellier, A., Zhao, X., Ruiz-Feria, C. (2007) Cecal populations of lactobacilli and bifidobacteria and *Escherichia coli* populations after in vivo *Escherichia coli* challenge in birds fed diets with purified lignin or mannanoligosaccharides. *Poultry science* 86, 2509-2516.
- Bozkurt, M., Aysul, N., Küçükylmaz, K., Aypak, S., Ege, G., Catli, A., Ak it, H., Çöven, F., Seyrek, K., Çınar, M. (2014) Efficacy of in-feed preparations of an anticoccidial, multienzyme, prebiotic, probiotic, and herbal essential oil mixture in healthy and *Eimeria* spp.-infected broilers. *Poultry science* 93, 389-399.
- Calik, A., Ergün, A. (2015) Effect of lactulose supplementation on growth performance, intestinal histomorphology, cecal microbial population, and short-chain fatty acid composition of broiler chickens. *Poultry science* 94, 2173-2182.
- Callaway, T., Edrington, T., Anderson, R., Harvey, R., Genovese, K., Kennedy, C., Venn, D., Nisbet, D. (2008) Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. *Animal Health Research Reviews* 9, 217-225.
- Chae, B., Lohakare, J., Moon, W., Lee, S., Park, Y., Hahn, T.W. (2006) Effects of supplementation of β -glucan on the growth performance and immunity in broilers. *Research in veterinary science* 80, 291-298.
- Corp, I. (2011) IBM SPSS statistics for windows, version 22.0. IBM Corp Armonk, NY.
- Cox, C., Stuard, L., Kim, S., McElroy, A., Bedford, M., Dalloul, R. (2010) Performance and immune responses to dietary β -glucan in broiler chicks. *Poultry science* 89, 1924-1933.
- Dizaji, B.R., Hejazi, S., Zakeri, A. (2012) Effects of dietary supplementations of prebiotics, probiotics, synbiotics and acidifiers on growth performance and organs weights of broiler chicken. *Eur. J. Exp. Biol* 2, 2125-2129.
- Fritts, C., Kersey, J., Motl, M., Kroger, E., Yan, F., Si, J., Jiang, Q., Campos, M., Waldroup, A., Waldroup, P. (2000) *Bacillus subtilis* C-3102 (Calsporin) improves live performance and microbiological status of broiler chickens. *The Journal of Applied Poultry Research* 9, 149-155.
- Fuller, R. (1989) Probiotic in man and animals: A rev. *J. Appl. Bacteriol* 90, 352.
- Gaggia, F., Mattarelli, P., Biavati, B. (2010) Probiotics and prebiotics in animal feeding for safe food production. *International journal of food microbiology* 141, S15-S28.
- Ganan, M., Silván, J.M., Carrascosa, A.V., Martínez-Rodríguez, A.J. (2012) Alternative strategies to use antibiotics or chemical products for controlling *Campylobacter* in the food chain. *Food Control* 24, 6-14.
- Hajati, H., Rezaei, M. (2010) The application of prebiotics in poultry production. *International Journal of Poultry Science* 9, 298-304.
- Hashim, M., Al-Ajeeli, M., Abdaljaleel, R., Alsadwi, A., Jameel, Y., Leyva-Jimenez, H., Haq, A., Corley, J., and Bailey, C.A. (2016) Performance of broilers fed diets supplemented with two yeast cell wall strains using two feeding strategies, In: *Poultry Science Association 105th Annual Meeting Abstracts*, p. 148.
- Hofacre, C., Mathis, G., Quiroz, M. (2005) Natural alternatives to prevent necrotic enteritis. *Int. Poult. Prod* 13, 7-9.
- Hume, M. (2011) Historic perspective: prebiotics, probiotics, and other alternatives to antibiotics. *Poultry science* 90, 2663-2669.
- Huyghebaert, G., Ducatelle, R., Van Immerseel, F. (2011) An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary Journal* 187, 182-188.
- Jameel, Y.J. (2008) The Effect of Adding Thyme vulgaris and Cinnamomum zeylanicum on production performance and some blood traits in broiler chicken, College of Veterinary Medicine, University of Baghdad.
- Jameel, Y.J., Abed, A., Al-Shimmary, F. (2014) Influence of Adding Garlic and Thyme and their Combination on Immune Response and Some Blood Parameters in Broiler. *Scientia* 2, 102-106.
- Kim, G.-B., Seo, Y., Kim, C., Paik, I. (2011) Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poultry Science* 90, 75-82.
- Lipke, P.N., Ovalle, R. (1998) Cell wall architecture in yeast: new structure and new challenges. *Journal of bacteriology* 180, 3735-3740.
- Loh, T.C., Choe, D.W., Foo, H.L., Sazili, A.Q., Bejo, M.H. (2014) Effects of feeding different postbiotic metabolite combinations produced by *Lactobacillus plantarum* strains on egg quality and production performance, faecal parameters and plasma cholesterol in laying hens. *BMC veterinary research* 10, 149.
- Mookiah, S., Sieo, C.C., Ramasamy, K., Abdullah, N., Ho, Y.W. (2014) Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. *Journal of the Science of Food and Agriculture* 94, 341-348.
- Mountzouris, K. (2014) Probiotics as alternatives to antimicrobial growth promoters (AGPs) in broiler nutrition: modes of action and effects on performance. In 'Probiotics in poultry production concepts and

applications'. (Ed. WHA Abdelrahman) pp. 129–157. 5m Publishing Ltd: Sheffield, UK.

Munyaka, P., Echeverry, H., Yitbarek, A., Camelo-Jaimes, G., Sharif, S., Guenter, W., House, J., Rodriguez-Lecompte, J. (2012) Local and systemic innate immunity in broiler chickens supplemented with yeast-derived carbohydrates. *Poultry science* 91, 2164-2172.

Park, J., Kim, I. (2014) Supplemental effect of probiotic *Bacillus subtilis* B2A on productivity, organ weight, intestinal *Salmonella* microflora, and breast meat quality of growing broiler chicks. *Poultry science*, PS3818.

Patterson, J., Burkholder, K. (2003) Application of prebiotics and probiotics in poultry production. *Poultry science* 82, 627-631.

Pourabedin, M., Xu, Z., Baurhoo, B., Chevaux, E., Zhao, X. (2014) Effects of mannan oligosaccharide and virginiamycin on the cecal microbial community and intestinal morphology of chickens raised under suboptimal conditions. *Canadian journal of microbiology* 60, 255-266.

Refstie, S., Baevefjord, G., Seim, R.R., Elvebø, O. (2010) Effects of dietary yeast cell wall β -glucans and MOS on performance, gut health, and salmon lice resistance in Atlantic salmon (*Salmo salar*) fed sunflower and soybean meal. *Aquaculture* 305, 109-116.

Ricke, S. (2015) Potential of fructooligosaccharide prebiotics in alternative and nonconventional poultry production systems. *Poultry science* 94, 1411-1418.

Santin, E., Maiorka, A., Macari, M., Grecco, M., Sanchez, J., Okada, T., Myasaka, A. (2001) Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. *The Journal of Applied Poultry Research* 10, 236-244.

Schumann, R. (2002) Clinical Medical and technological properties of lactulose. *An Update. Eur. J. Nutr* 41, 117-125.

Steiner, T. (2006) *Managing gut health: natural growth promoters as a key to animal performance*. Nottingham university press.

Sugiharto, S. (2016) Role of nutraceuticals in gut health and growth performance of poultry. *Journal of the Saudi Society of Agricultural Sciences* 15, 99-111.

Teo, A., Tan, H.-M. (2007) Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with *Bacillus subtilis* PB6 (CloSTAT). *The Journal of Applied Poultry Research* 16, 296-303.

Tuohy, K.M., Probert, H.M., Smejkal, C.W., Gibson, G.R. (2003) Using probiotics and prebiotics to improve gut health. *Drug discovery today* 8, 692-700.

Yalçinkaya, ., Guengoer, T., Ba alan, M., Erdem, E., (2008) Mannan oligosaccharides (MOS) from *Saccharomyces cerevisiae* in broilers: Effects on performance and blood biochemistry. *Turkish Journal of Veterinary and Animal Sciences* 32, 43-48.

Yang, Y., Iji, P., Kocher, A., Mikkelsen, L., Choct, M., (2007) Effects of mannanoligosaccharide on growth performance, the development of gut microflora, and gut function of broiler chickens raised on new litter. *The Journal of Applied Poultry Research* 16, 280-288.

Zhao, P., Wang, J., Kim, I. (2013) Effect of dietary levan fructan supplementation on growth performance, meat quality, relative organ weight, cecal microflora, and excreta noxious gas emission in broilers. *Journal of animal science* 91, 5287-5293.

Zhao, P.Y., Li, H.L., Mohammadi, M., Kim, I.H. (2016) Effect of dietary lactulose supplementation on growth performance, nutrient digestibility, meat quality, relative organ weight, and excreta microflora in broilers. *Poult Sci* 95, 84-89.