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Probiotic in broiler diet: effect on performance parameters

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Abstract

In this experiment, the performance of broilers, supplemented with a commercial inactivated probiotics, was evaluated. Four hundred and fifty day old broiler chickens were allocated into 3 groups with 6 replicates each. In group 1, feed was supplemented with 500g/ton of a heat-inactivated probiotic containing *Lactobacillus spp*, *Bifidobacterium animalis*, *Pediococus acidilactici* and *Enterococcus faecium*. In group 2, the feed was supplemented with 500g/ton of the same probiotic without the heat-inactivating process (commercial product). In group 3, the feed was supplemented with zinc bacitracin at 100 ppm. During the pre-initial phase, group 2 had significantly higher feed intake compared to the other groups. In the finisher stage, feed intake was significantly low in group 2 and weight gain was significantly high in the same group. From day 1-21, the FCR was significantly high in group 2. Regular and inactivated probiotics had superior effect compared to the group containing 100ppm of zinc bacitracin.

Keywords: Broilers; feed conversion; probiotics

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Introduction

Probiotics are defined as live microbial supplements which are able to exert beneficial actions in the target host by improving its intestinal microbial balance (Fuller, 1989). In farm animals, probiotics have been extensively tested under different experimental and commercial scenarios. Benefits of probiotics in performance parameters (body weight and feed conversion) and gastrointestinal health of chickens have been extensively reported in the literature (Jarquin et al., 2007; Talebi et al., 2008; Ignatova et al., 2009). The actions of probiotics are thought to be derived from their ability to compete directly with pathogens for nutrients and binding sites, production of substances with antimicrobial activity, aggregation of pathogens limiting their binding activity and stimulation of the immune system (Pascual et al., 1999; Ibnou-Zekri et al., 2002; Fayol-Messaoudi et al., 2005). Effects of probiotics in growth promotion are often explained in a similar manner: by the action of the probiotics within the intestinal tract of the animal would have less challenge from pathogenic bacteria and their toxins. Consequently, less energy is utilized to mobilize immune cells to fight pathogens and fewer resources are needed to repair damaged tissue. There is increasing evidence suggesting that some of the modes of action of probiotics are not related to their viability. For example, experimental colitis in mice can be alleviated using either probiotics or their isolated DNA molecules, indicating that the viability of probiotics might not be a requirement at least in some of the probiotics targeted gastrointestinal disorders (Rachmilewitz et al., 2004). Moreover, it has been suggested that the definition of probiotics should be expanded from considering only the effects of "live microbial supplements" to a definition including the effects derived from "the components of microbial cells" (Salminen et al., 1999). In the current experiment, a heat-inactivated Poltryu Star®, a well defined synbiotic (a mixture of probiotics and prebiotics) was used to conduct a performance experiment in broiler chickens. The aim of the study

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was to compare the results of the standard product, its inactivated form and a commonly used growth promoter (zinc bacitracin) in absence of a known pathogenic challenge.

Materials and Methods

Four hundred and fifty day-old broiler males were purchased from the local hatchery where they were vaccinated against Marek's and Newcastle diseases. Birds were weighed, wing tagged and randomly distributed into three groups with six replicates of 25 birds each. Birds from each replica were placed on clean wood shavings in floor pens equipped with tubular feeders, drinkers and incandescent lamps as heat source. Birds received 24 h of light per day. Birds received feed and water *ad libitum* throughout the experimental period. Feed was formulated to meet or exceed the nutrient requirements of the NRC (1994). The experimental period was divided into four phases: pre-initial, 1 to 7 days; initial, 8 to 21 days; growth, 22 to 35 days; and finisher, 37 to 40 days. Experimental diets of all phases were isoprotein (22.04, 20.79, 19.41 and 18.03% crude protein), and isocaloric (2.900, 3.000, 3.100, 3.150 kcal/kg ME) and given to the birds in a mashed form. The basal diets were supplemented with regular Poultry Star® synbiotic (a mixture of prebiotic and probiotic bacteria procured from local market which is composed of *Lactobacillus spp, Bifidobacterium animalis, Pediococus acidilactici and Enterococcus faecium* with a total of 10^{11} CFU/Kg of product) or with heat-inactivated Poultry Star®. Diet supplementation with zinc bacitracin was included as positive control.

Inactivation of the probiotic 500g of the pre-mixture of the probiotic strains of Poultry Star® (feed version) was suspended in 3L of tap water and autoclaved for 20 min at 123°C. After cooling, the probiotic solution was freeze dried and cultured to verify the effectiveness of the inactivation process. The dried inactivated probiotic was then mixed with feed at the same rate than the regular probiotic.

Group 1: Inactivated Poultry Star®; 500 g/ton of feed.
Group 2: Poultry Star®; 500 g/ton of feed.
Group 3: Zinc bacitracin; 100 ppm

Birds were weighed at 1, 7, 21, 35 and 40 days of age. Feed intake was calculated as the difference between the amount of feed provided and the refusals after the end of each period. Feed intake and feed conversion data were corrected for mortality. Feed conversion per pen was calculated using the feed intake and body weight gain at the end of each period. Data were analyzed using the R statistical software with one way analysis of variance. Differences were declared with $P<0.05$ and means were separated using the Tukey test.

Results

From day 1 to 7, there was a significantly higher feed intake in the group that did not consume probiotics (group 3). Birds in group 2 had the lowest numerical feed intake. However, in group 2, the weight gain was numerically the highest among the three groups. Feed conversion ratio was not statistically different between the groups (Table 1). From day 8 to 21, the groups 1 and 2 had lower feed conversion ratio compared to group 3. During this period there were no differences between the groups in feed intake or weight gain (Table 1). From day 22 to 36, there was no significant difference between groups in any of the productive parameters measured in this experiment (Table 1). From days 37 to 40, birds from the group 3 had a significantly lower weight gain and a significantly higher feed conversion compared to the birds in groups 1 and 2 (Table 1).

Table 1: Effect of the use of regular probiotics, inactivated probiotics and zinc bacitracin on feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) of broiler chicks in different periods of age

Groups (n=150)	Age (days)	Feed intake (g/broiler)	Weight gain (g/broiler)	Feed conversion ratio (g/g)
Probiotic inactivated	1 to 7	177.04[b]	145.17	1.22
Probiotic regular	1 to 7	173.76[a]	149.97	1.16
Zinc bacitracin	1 to 7	182.45[c]	147.48	1.24
Probiotic inactivated	8 to 21	1137.99	742.43	1.53[b]
Probiotic regular	8 to 21	1137.29	745.09	1.53[b]
Zinc bacitracin	8 to 21	1158.51	710.02	1.63[a]
Probiotic inactivated	22 to 36	2052.24	1153.65	1.78
Probiotic regular	22 to 36	208020	1201.31	1.73
Zinc bacitracin	22 to 36	2003.51	1137.78	1.76
Probiotic inactivated	37 to 40	852.75[b]	1153.65[b]	1.78
Probiotic regular	37 to 40	868.97[c]	1201.31[a]	1.73
Zinc bacitracin	37 to 40	827.77[a]	1137.78[c]	1.76

[a,b]Means followed by different superscript letters within the same age group are significantly different ($P<0.05$)

Table 2: Effect of probiotics, inactivated probiotics and zinc bacitracin on feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) of broiler chicks of broiler chicks in different periods of age

Groups (n=150)	Age (days)	Feed intake (g/broiler)	Weight gain (g/broiler)	Feed conversion ratio (g/g)
Probiotic inactivated	1 to 21	1317.22	879.81	1.50[b]
Probiotic regular	1 to 21	1294.67	887.88	1.45[a]
Zinc bacitracin	1 to 21	1342.00	857.50	1.57[c]
Probiotic inactivated	22 to 40	2912.10	1592.10	1.83
Probiotic regular	22 to 40	2955.88	1615.75	1.83
Zinc bacitracin	22 to 40	2833.64	1531.11	1.83
Probiotic inactivated	Final (1-40)	4434.76	2471.91	1.79
Probiotic regular	Final (1-40)	4444.81	2503.63	1.78
Zinc bacitracin	Final (1-40)	4385.92	2388.61	1.84

a, b Means followed by different superscript letters within the same age group are significantly different (P<0.05).

From days 1 to 21, FCR in birds from group 2 was significantly lower than birds in group 3. There were no statistical differences in feed intake or weight gain between groups (Table 2). From day 22 to 40, there were no statistical differences between groups in the productive parameters evaluated in this study (Table 2).

In this trial, data were arranged in two ways. It was possible to observe more statistical differences favouring the group of birds from group 2 when the data was arranged following the diet changed. In general, birds fed regular and inactivated probiotics performed similarly. The overall productive parameters are presented in Table 2. No statistical differences were detected when analyzing the complete data set; however, the numerical differences observed in weight gain and feed conversion may be of interest.

Discussion

Probiotics are live bacteria that confer benefits to their hosts by a variety of mechanisms. Traditionally, direct inhibition of pathogens by metabolically active bacteria has been used to explain their efficacy as natural growth promoters (Fuller, 1989; Pascual et al., 1999; Ibnou-Zekri et al., 2002; Fayol-Messaoudi et al., 2005). In theory, by competing with pathogenic bacteria, it is possible to minimize damage exerted to the intestinal mucosa by improving nutrient absorption and reducing the need of spending resources on tissue healing. Regular and inactivated probiotics have demonstrated their efficacy reducing the inflammation and epithelial necrosis induced in experimental inflammatory colitis (Rachmilewitz et al., 2004). Protection of inactivated probiotics seems to be derived from the interaction of specific sequences of their DNA with the host's Toll-Like-Receptor 9 (TLR-9) molecules. This interaction induces the production of anti-inflammatory molecules in mice like interferon (IFN) (Katakura et al., 2005). Gastric and intestinal lesions derived from group with indomethacin were ameliorated in rats receiving both regular and inactivated probiotics compared to a control receiving neither. Interestingly, the indomethacin-induced neutrophil infiltration of the gastrointestinal mucosa was also decreased with the use of live and dead probiotics (Laudanno et al., 2006). Adhesion of probiotics to intestinal mucus has also been demonstrated for inactivated probiotics.

Adhesiveness of probiotics to intestinal mucus varies with the method of inactivation and it is normally reduced in inactivated compared to viable bacteria. However, in some selected strains of probiotics, an increased adherence index has been achieved after the inactivation when compared to the live control (Ouwehand et al., 2000). In the present study, we demonstrated that at least some of the benefits of inactivated probiotics observed in mice are reproducible in broiler chickens. Since broilers fed inactivated probiotic performed similar to birds fed the regular probiotic it is likely that not all growth promoting effects derived from probiotics are due to the metabolic functions of probiotic bacteria. In the present study, the effects of the inactivated probiotic group may be confounded with the effect of prebiotics which were probably not affected by the inactivation process. It is a possibility that the benefits derived from the prebiotics was equal to the benefits provided by the mixture of probiotics and prebiotics. The mode of action of antibiotics used as growth promoters is currently unknown. However, it is widely accepted that growth promoters "somehow" enhance performance in farm animals like poultry and swine. Several theories have been proposed to explain the efficacy of antibiotic growth promoters. Most of these theories involve direct effect of the antibiotics on the intestinal microflora (Dibner and Richards, 2005). In addition to those theories, a non-antibiotic mediated mode of action has also been proposed for antibiotics used as growth

promoters (Niewold, 2007). This is due to the fact that the concentration of antibiotics when used as growth promoters are not sufficient to reach the minimum inhibitory concentration (concentration of antibiotic needed to inhibit the growth of bacteria *in vitro*) of common pathogens. In addition, it is known that antibiotics given at low doses can be uptaken by immune cells of the intestinal mucosa. Within these cells, the antibiotics can exert an anti-inflammatory action by increasing the stimuli needed to degranulate heterophils reducing self-inflicted tissue damage due to exaggerated immune response to commensal microflora and feed antigens (Roura et al., 1992; Niewold, 2007).

Even though the biological action of inactivated probiotics has been demonstrated under certain scenarios it is doubtful that dead probiotics will perform as well as live probiotic under all scenarios. It is likely that the "old fashioned" modes of action of probiotics like direct anti-pathogenic action may also play important roles under a pathogenic challenge. For example, live probiotics worked better than inactivated probiotics in a challenge trial with *Edwarsiella tarda* in tilapia (Taoka et al., 2006). A natural enzymatic and bacterial degradation is expected after bacteria are inactivated and thus manufactures may still be forced to supply the product in a biologically active form to ensure appropriate shelf life.

Conclusion

Under the conditions of the current experiment, birds fed inactivated and regular synbiotics had similar performance parameters. Data presented in this experiment encourage studies evaluating the benefits of non-coated synbiotics in high temperature pelleted feed or even in feed which is being extruded or combined with antibiotics.

References

Dibner, J.J. and Richards, J.D. 2005. Antibiotic growth promoter in agriculture: history and mode of action. *Poultry Science,* 84: 634-643.

Fayol-Messaoudi, D., Berger, C.N., Coconnier-Polter, M.H., Moal, V.L. and Servin, A.L. 2005. Ph-, lactic acid- and non-lactic acid-dependent activities of probiotic Lactobacilli against *Salmonella enterica* serovar Typhimurium. *Applied Environmental Microbiology,* 71(10): 6008-6013.

Fuller, R. 1989. Probiotics in man and animals. *Journal of Applied Bacteriology,* 66: 365-378.

Ibnou-Zekri, N., Blum, S., Schiffrin, E.J. and VonDer, Weid T. 2002. Divergent patterns of colonization and immune response elicited from two intestinal Lactobacillus strains that display similar properties *in vitro*. *Infection and Immunity*, 71(1): 428-436.

Ignatova, M., Sredkova, V. and Marasheva, V. 2009. Effect of dietary inclusion of probiotic on chicken performance and some blood indices. *Biotechnology in Animal Husbandry*, 25: 1079-1085.

Jarquin, R.L., Nava, G.M., Wolfenden, A.D., Donoghue, A.M., Hanning, I., Higgins, S.E. and Hargis, B.M. 2007. The evaluation of organic acids and probiotic cultures to reduce *Salmonella enteritidis* horizontal transmission and crop infection in broiler chickens. *International Journal of Poultry Science,* 6(3): 182-186.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. Ed. National Academy Press, Washington, DC.

Niewold, T.A. 2007. The non-antibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. *Poultry Science*, 86: 605-609.

Ouwehand, A.C., Tölkkö, S., Kulmala, J., Salminen, S. and Salminen, E. 2000. Adhesion of inactivated probiotic strains to intestinal mucus. *Letters of Applied Microbiology,* 31: 82-86.

Pascual, M., Hugas, M., Badiola, R.I., Monfort, J.M. and Garriga, M. 1999. Lactobacillus salivarius CTC2197 prevents *Salmonella enteritidis* colonization in chickens. *Applied Environmental Microbiology*, 65: 4981-4986.

Rachmilewitz, D., Katakura, K., Karmeli, F., Hayashi, T., Reinus, C., Rudensky, B., Akira, S., Takeda, K., Lee, J. and Takabayashi, K. 2004. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology,* 126(2): 520-528.

Roura, E., Homedes, J. and Klassing, K.C. 1992. Prevention of immunologic stress contributes to the growth-permittiong ability of dietary antibiotics in chicks. *Journal of Nutrition,* 122: 2383-2390.

Salminen, S., Ouwehand, A., Benno, Y. and Lee, Y.K. 1999. Probiotics: how should they be defined? *Trends. Food Science Technology*, 10: 107-110.

Talebi, Amirzadeh, A., Mokhtari, B. and Gahri, H. 2008. Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. *Avian Pathology*, 27(5): 509-512.

Taoka, Y., Maeda, H., Jo, J.Y., Kim, S.M., Park, S.I., Yoshikawa, T. and Sakata, T. 2006. Use of live and dead probiotic cells in tilapia *Oreochromis niloticus*. *Fisheries* Science, 72: 755-766.