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Combined use of probiotics and other active ingredients in broiler production during free antibiotic period - an update review

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Abstract

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Probiotics has gaining a great attention from broiler producers during the post-antibiotic era. The application of such bioactive substance is actually expected to safely substitute the use of antibiotic growth promoters in broiler production. Yet, their efficacy as in-feed antibiotic substitute for broiler chickens is erratic. The combined use of probiotics and other active compounds, such as prebiotics, phytobiotics, enzymes and organic acids, is subjected to improve the potential of probiotic in promoting the growth and wellbeing of broiler chickens. The synergistic or complementary effect between probiotics and other active ingredient would be greatly beneficial for the production and health of broilers more than either supplement administrated on its own. This review aimed to update the use of probiotics simultaneously with other active compounds in broiler production. The possible synergistic effect between probiotics and its counterpart on broiler production is also investigated in this present review. Overall, some studies reported that the combined administration of probiotics and other active ingredients resulted in synergistic or complementary effects, while other studies pointed out no mutual supportive effect between probiotics and its counterpart on broiler production.

Keywords: broiler; enzyme; organic acid; phytobiotics; prebiotic; probiotics

Introduction

Antibiotic growth promoters for broiler production are no longer used in various part of the world. This is due to the concerns about the presence of antibiotic residues in broiler chicken meat so that it can endanger human health. Indeed, the retraction of antibiotic growth promoters from broiler rations has encouraged the nutritionists to look for the safe and natural substitute for antibiotic growth promoters for broiler production (Dittoe et al., 2018). The substitute for antibiotic growth promoters is inevitability for modern broiler strains as the withdrawal of in-feed antibiotics is attributable to the increased rate of mortality and morbidity in broiler farms. Probiotic is one of the most popular alternatives for in-feed antibiotic for broiler production. This is a living microorganism, which in a certain amount, can improve the balanced intestinal microbial ecosystem, immune development and hence health and production parameters of host (Sugiharto, 2016). Other active ingredient that has usually been used in broiler production is prebiotics. Prebiotics may be defined as the substances that are resistant to the digestive activities of host but can be utilized as the substrates for the microorganisms, and thus conferring the beneficial impacts on health and production parameters of the host (Markowiak & Śliżewska, 2018). Phytobiotics or phytochemical compounds represent a broad variety of plant-derived compounds including herbs, essential oils and oleoresins (Gheisar & Kim, 2018). Following the withdrawal of in-feed antibiotics, phytobiotics has gained more attention to maintain the health status and growth potential of broilers. In general, phytobiotics can be

introduced to the diets of broilers by stimulating the immune response, balancing the intestinal microbial ecosystem, improving feed characteristics, promoting the production efficiency and enhancing the quality of broiler meats (Sugiharto, 2016; Gheisar & Kim, 2018). Enzyme is another bioactive ingredient that is usually supplemented into diets to maximize the growth rate of broilers. The enzyme supplementation is attributed to the improvement in nutrient digestibility and utilization, and hence the growth capacity of broilers (Singh et al., 2019). Organic acid, which is an organic substance with acidic characteristic, is other active compound that is promising to replace in-feed antibiotics in broiler husbandry. Dittoe et al. (2018) revealed that organic acid can alter the pH of gastrointestinal tract and thereby improving the microbial composition and morphology of the gastrointestinal tract of broilers. The latter condition could therefore improve the immune defense and digestive and absorptive capacities of the intestine of broilers (Sugiharto, 2016; Dittoe et al., 2018).

During the post-antibiotic era, probiotics have widely been used in broiler production. Yet, various reports showed that the efficacy of probiotics in altering the role of in-feed antibiotics is inconsistent. Hussein et al. (2020a) reported the positive effect of *Bacillus*-based probiotic on the weight of broilers at day 35 of age. Also, Machado et al. (2020) observed that the probiotic (not specified) treatment increased the growth performance of broilers as compared to control. In contrast to these above studies, Bai et al. (2013) documented that dietary supplementation of probiotic (*Saccharomyces cerevisiae* and *Limosilactobacillus fermentum*) did not exert any substantial impact on the final body weight of broilers. Likewise, Olnood et al. (2015) observed no influence of *Lactobacillus*-based probiotic on broiler growth. Several arguments may explain the inconsistent data on growth of broilers due to probiotic administration. Blajman et al. (2014) suggested that variations in weight gain of broilers with dietary administration of probiotic could be attributable to the disparities in species/strains of probiotic microorganisms, level or number of colonies of probiotic microorganism, routes of delivery (either through feed or water) and duration of the broiler experiment. Also, the composition of feeds and the conditions of experiment (e.g., hygiene level, stress and ages and strains of broilers) may assign the impact of probiotics on the production performance and wellbeing of broilers.

Improving the effectiveness of probiotics as the alternative to antibiotic growth promoters is beneficial for the sustainable broiler production during the post-antibiotic period. Recently, there has been a growing interest in combining of probiotics and other functional ingredients to further improve the efficacy of probiotics in promoting the productive and health performance of broilers more than either additive applied on its own (Sugiharto, 2016; Rodjan et al., 2018; Sugiharto et al., 2019). It is expected that the combined application of probiotics and other active compounds can exert a synergistic or complementary effect to each other (Bolivar et al., 2018), and thus further improve the efficacy of probiotics as the substitute to antibiotic growth promoters for broilers. The current review aimed to update the information regarding the use of probiotics simultaneously with other active compounds in broiler production. The review also investigated the possible synergistic or complementary impact of probiotics and its counterpart on production of broiler chickens.

Combined Use of Probiotics and Prebiotics in Broiler Production

Probiotics as well as prebiotics have widely been used in broiler production to maintain the maximum growth and health of poultry during post-antibiotic era. Apart from its beneficial impacts, the application of probiotics or prebiotics separately has frequently been reported to be inconsistent in supporting the growth and health performances of broilers (Sugiharto, 2016; Markowiak & Śliżewska, 2018). The survival and stability of probiotics in the gut are the key factors determining the effectiveness of probiotics in exerting positive influences on broilers. While, the efficacy of prebiotics on broiler production and wellbeing greatly depend on the compound specificity, doses and time of administration of prebiotics (Markowiak & Śliżewska, 2018). Indeed, prebiotics can provide energy and nutrients for probiotic microorganisms particularly in the intestine of broilers. For this reason, the simultaneously use of probiotics and prebiotics could be directed to promote the survival and stability of probiotics in the gastrointestinal tract of broilers (Markowiak & Śliżewska, 2018). In general, the combined application of probiotics and prebiotics is called as synbiotics.

The use of synbiotics (a mixture of probiotics and prebiotics) in broiler production has been reported by several authors. Tayeri et al. (2018) revealed that dietary inclusion of synbiotics produced better growth rate, feed conversion and carcass characteristic of broiler chickens relative to administration of probiotics (mixture of *Lactiplantibacillus plantarum* subsp. *plantarum*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lacticaseibacillus rhamnosus*, *Enterococcus faecium*, *Aspergillus oryzae*, *Bifidobacterium bifidum*, *Streptococcus salivarius* subsp. Hermophilus and *Candida pintolopesii*) or prebiotics

(polysaccharides derived from *Astragalus membranaceus*) alone. Dietary administration of synbiotics also generated more superior immune system of broiler than that of individual administration of probiotic or prebiotics (Tayeri et al., 2018). In agreement, Kamel & Mohamed (2016) reported that dietary supplementation of synbiotics (Baymix® GrobigTM + Cel-Max dryTM) resulted in more superior final body weight and feed efficiency of Ross broilers than that of probiotics (Baymix® GrobigTM) or prebiotics (Cel-Max dryTM) alone. The eminent growth performance was also reported by Abdel-Hafeez et al. (2017) in synbiotics (half amount of probiotics and prebiotics) fed broilers when compared with that of fed probiotics (*Bacillus licheniformis* and *Bacillus subtilis*) or prebiotics (phosphorylated mannan-oligosaccharides isolated from the cell wall of *Saccharomyces cerevisiae*) alone. Likewise, Kırkpınar et al. (2018) noticed that combination of probiotics (*Streptococcus salivarius* subsp. thermophilus, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactiplantibacillus plantarum* subsp. *plantarum*, *Lacticaseibacillus rhamnosus*, *Enterococcus faecium*, *Bifidobacterium bifidum*, *Asperigillus oryzae* and *Candida pintoloppesii*) and prebiotics (*Aspergillus* meal) resulted in better live weight gain in broiler chickens relative to single administration of probiotics or prebiotics. Dizaji et al. (2012) also documented that dietary supplementation of synbiotics resulted in greater weight of broiler at 42 days of age as compared to that of probiotics supplementation. Higher body weight was also shown by Das et al. (2016) when feeding commercial synbiotics (Nutriferm + Protexin) to broiler chickens when compared with feeding commercial probiotics (Protexin). Moreover, Al-Sultan et al. (2016) reported that dietary synbiotics (blend of *Enterococcus faecium* and oligosaccharides) supplementation resulted in more superior live weight at day 42 relative particularly to commercial prebiotic (mannan-oligosaccharides) supplementation. Compared to the individual administration of commercial probiotic (*Saccharomyces cerevisiae*-based probiotics) or prebiotic (mannan-oligosaccharides), feeding synbiotics (*Saccharomyces cerevisiae* and mannan-oligosaccharides) showed more improved feed conversion ratio of broilers in the study of Abdel-Raheem et al. (2012). Wang et al. (2016) further reported that dietary inclusion of probiotic (*Bacillus subtilis*) in combination with prebiotic (mannan-oligosaccharides and β-glucans) delivered higher final body weight and feed efficiency compared to the use of prebiotic alone. Moreover, Sohail et al. (2013) documented that the combination of probiotic mixture (*Lactiplantibacillus plantarum* subsp. *plantarum*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus*, *Streptococcus thermophilus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Aspergillus oryzae* and *Candida pintolopesii*) and prebiotic (mannan-oligosaccharide) resulted in greater weight gain as compared to the supplementation of probiotic or prebiotic separately to broilers exposed to heat stress at 42 days of age.

The synergistic improvement in digestive and absorptive capacity of broilers by probiotic and prebiotic is most likely be attributed to the further improved nutrient utilization and hence growth rate of broilers. Beski & Al-Sardary (2015) reported that dietary use of synbiotics (blends of *Lactobacillus acidophilus*, *Bifidobacterium*, *Bacillus subtilis* and *Saccharomyces cervisiae* and fructooligosaccharide derived from Jerusalem artichoke) resulted in higher villus height of ileum of broilers when compared with that of probiotics (*Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium* and *Saccharomyces cervisiae*) alone. Also, Al-Baadani et al. (2016) showed that synbiotics treatment increased jejunal villus height and surface area of broiler challenged with *Clostridium perfringens* when compared with that of probiotics (*Bacillus subtilis*) or prebiotics (mannan-oligosaccharides and β-1, 3 glucanes and derived from the cell membrane of *Saccharomyces cerevisiae*) individually. The latter condition may then contribute to the increased absorptive capacity of chickens. This inference was supported by Al-Sultan et al. (2016) showing the synergistic positive effect of probiotic (*Enterococcus faecium*) and prebiotics on villus height, crypt depth, villus height to crypt depth ratio along the small intestinal segments of broilers relative to probiotic or prebiotic alone. In agreement, Çalık et al. (2017) reported synergistic effect between probiotic *Paenibacillus xylanexedens* and prebiotic lactulose in enhancing the villus height of broilers, relative to treatment with probiotic alone. The synergistic effect of probiotic and prebiotic in improving the intestinal microbial ecosystem may also contribute to the further promotion of health status and thus growth rate of broilers. In this case, the synergistic positive effect of probiotic (*Enterococcus faecium*) and prebiotics on total bacteria and lactose fermenter bacteria along the intestine of broiler relative to probiotic or prebiotic alone was demonstrated by Al-Sultan et al. (2016). The further improvement on immune properties of broilers may also be associated with the improved health of chickens, as Ghasemi & Taherpour (2013) reported that synbiotics (combination of *Enterococcus faecium* NCIMB 10415 and oligosaccharides extracted from chicory root) treatment resulted in better immune response (serum anti- sheep red blood cell [SRBC] antibody titers) of broilers at 41 days of age, when compared with individual probiotic (*Enterococcus faecium* NCIMB 10415) or prebiot-

ic (oligosaccharides extracted from chicory root) treatment.

Beyond of the above beneficial effects, the mixture of probiotic and prebiotic may not always result in complementary effect on growth and health of broilers. In their report, Ghasemi & Taherpour (2013) did not show any further improvement to probiotic or prebiotic effect on productive parameters and intestinal histology of broiler chickens when these active components were combined. Also, Çalık et al. (2017) reported no synergistic effect of probiotic *Paenibacillus xylanexedens* either with prebiotic inulin or prebiotic lactulose in term of growth performance of broilers. Mohammed et al. (2016) also did not see any synergistic influence of probiotic and herbal-based prebiotics on the growth achievement of broilers. Moreover, Mookiah et al. (2014) did not see any synergistic impact between probiotics and prebiotics in growth rate and intestinal ecology of broilers. The absent synergistic impact between probiotic (*Lactobacillus acidophilus, Pediococcus acidilactici, Lacticaseibacillus casei, Bacillus subtilis* and *Saccharomyces boulardii*) and probiotic (mannan-oligosaccharide) was also reported by Saiyed et al. (2015) on growth performance of broilers. In addition, Murate et al. (2015) found that synbiotics (85% of the probiotic + 15% of the prebiotic) did not affect *Salmonella enterica* serovar Enteritidis infection in broilers as compared to probiotics (*Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Bacillus subtilis*, *Bifidobacterium longum* and *Enterococcus faecium*) or prebiotics (fructooligosaccharide, inulin, mannan-oligosaccharide and oligosaccharide) alone. Indeed, Abdel-Raheem et al. (2012) showed that feeding synbiotics (combination of *Saccharomyces cerevisiae* and mannan-oligosaccharides) decreased the numbers of *Lactobacilli* in duodenum of broilers as compared to prebiotic (mannan-oligosaccharides) administration alone. The inconsistent results regarding the combined use of probiotics and prebiotics could not be definitely explained. However, there are some sources of variations that can elucidate the above conditions, including the types, species and strains of microbes used as probiotics, the types of prebiotics, the doses of probiotics and prebiotics, the breeds or strains of broilers and other experimental conditions. In their study, Çalık et al. (2017) clarified that the disparity in outcomes can be due to the various prebiotics used in combination with probiotics. In such case, they pointed out that combination of *Paenibacillus xylanexedens* and lactulose resulted in higher villus height of broilers relative to the combination of *Paenibacillus xylanexedens* and prebiotic inulin or *Paenibacillus xylanexedens* alone. With regard to the effect of breeds and strains of broilers, Kamel & Mohamed (2016) documented that the effect of synbiotics on growth performance varied between Cobb and Ross broilers.

Combined Use of Probiotics and Phytobiotics in Broiler Production

Probiotics and also phytobiotics have been explored as the alternative for antibiotic growth promoters in broiler production for decades. Yet, the data regarding the effect of probiotics as well as phytobiotics seem to be variable, as some studies reported the positive while the other studies reported no effect (Sugiharto, 2016; Duskaev et al., 2018). Data obtained from the *in vitro* study (Prakasita et al., 2019) showed that herbal extract (i.e., red ginger [*Zingiber officinale* var. Rubrum], wild ginger [*Curcuma xanthorrhiza*] and turmeric [*Curcuma domestica*] possessed promoting effect on the growth of *Lactobacillus acidophilus* and *Levilactobacillus brevis*. Owing to this fact, the possible synergistic effect between probiotics and phytobiotics is therefore interesting to be further investigated (Ren et al., 2019). Indeed, the application of phytobiotics could be expected to further improve the growth- and health-promoting effect of probiotic *in vivo*. Ferdous et al. (2019) have recently reported that the combined use of commercial probiotics (Bio-Top®) and phytobiotics (Galibiotic®) resulted in better final body weight as compared especially to the use of phytobiotics (Galibiotic®) alone. Elkhouly et al. (2016) also revealed that combination of probiotic (*Pediococcus acidilactici*, *Acetobacter aceti*, *Pediococcus pentosaceus* and *Bacillus amyloliquefaciens*) and phytobiotics (Orego-stim® containing a- pinene, sabinene, camphene, B-pinene, Myrecene, aphellandrene, Limonene, a-terpinene, 1.8 cineole, B-Ocimene, Trpinolene, 1-Octn-3-o 1, trans-Sabinene hydrate, Linalool, Cis-sabinene hydrate, terpinrn-4ol, a-Terpineol, borneol, BBisabolene, carvacrol, y-terpinrnr, p- cymene and thymol) produced more superior growth performance than that of probiotic or phytobiotic alone to broilers infected with *Eimeria* spp. It is very likely that the use of probiotics in combination with phytobiotics further improves the gut ecology and thereby digestive functions of broiler intestine. This inference is supported by Ren et al. (2019) documenting the synergistic effect between probiotic (*Ligilactobacillus salivarius* and *Ligilactobacillus agilis*) and commercial phytobiotics (not specified) in improving the intestinal microbiota population of broilers. The latter report is actually supported by Prakasita et al. (2019) as mentioned above (regarding the stimulating effect of herbs on probiotic growth). In other studies, the combination of probiotic and phytobiotics further improved immune status (Elkhouly et al., 2016; Dong et al., 2019), which may then be attributed to the more allocation of energy for growth instead of for maintenance and recovery.

Different from the above studies, Hussein et al. (2020a) found no complementary effect of live probiotics (CloStat, *Bacillus subtilis*) and phytobiotics (Sangrovit Extra, sanguinarine and protopine) on the growth and carcass traits of broilers. In other study, Hussein et al. (2020b) also reported no symbiotic effect between probiotics (CloStat, *Bacillus subtilis*) and phytobiotics (Sangrovit Extra, sanguinarine and protopine) on the feed intake, final body weight and feed conversion ratio as well as intestinal histomorphometric traits of broilers challenged with *Clostridium perfringens*. In line with this, Alfaig et al. (2013) noticed no further influence on the carcass traits of broilers upon the use of combined probiotic *Bacillus subtilis* PB6 and thyme essential oil (*Thymus vulgaris* L.) compared to the single use of such probiotics and phytobiotics. In agreement, Duskaev et al. (2018) found no synergistic effect between probiotic (*Lactobacillus acidophilus* and *Bifidobacterium Adolescentis*) and phytobiotic (extract of *Quercus cortex*) on growth performance, immunity and health of broiler chickens. Dong et al. (2019) further reported that the use of probiotic (*Enterococcus faecalis*) in combination with phytobiotic (*Camellia oleifera* seed extract) did not exert synergistic impact on the growth rate of broilers challenged with *Escherichia coli* K88. It is very possible that variations in species, strains and doses of probiotics as well as the differences in types, sources and levels of phytochemical compounds may explain the divergent results regarding the use of probiotics simultaneously with phytobiotics on broiler chickens.

Combined Use of Probiotics and Enzymes in Broiler Production

Probiotic has been attributed to the improvement of gut microbial ecosystem and morphology, and, thus, intestinal digestive and absorptive functions of broilers. To maximize the digestive functions of the intestine, exogenous enzymes have commonly been incorporated to broiler feed (Sugiharto, 2016). Recently, there has been a great interest to combine probiotics and enzymes to exert further benefits than probiotics or enzymes alone. Earlier study by Momtazan et al. (2011) documented that a combination of probiotics (mixture of *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Bifidobacterium bifidum*, *Enterococcus faecium*) and enzyme complex (β-glucanase, α-amylase, cellulase, protease and lipase) improved the performance of broiler more than either supplement used on its own. In addition, Rahman et al. (2013) revealed that a combination of probiotics (Microguard®) and enzymes (Achmezyme®) produced better final body weight of broiler compared to single administration of probiotic or enzyme. Ali et al. (2018) also documented that a combination of probiotic (*Companilactobacillus farciminis* CNCM MA67/4R) and enzyme (endo-1, 4-β-xylanase, α-amylase and protease) resulted in higher final body weight compared to single administration of probiotic and enzyme. The further improvement in digestibility and nutrient utilization seems to be attributable to the enhanced growth rate of broiler treated with mixtures of probiotics and enzymes. In such case, Singh et al. (2019) found that the blends of probiotics (*Bacillus* sp.) and multi-enzymes (amylase, xylanase and protease) improved the nutrient utilization in broilers to a greater extent than by the single use of the additives. Moreover, the improved health status could also be attributed to the further improvement in growth performance of broilers treated with a mixture of probiotic and enzymes. Hosseini et al. (2017) documented that a mixtures of *Bacillus*-based probiotic and enzyme (carbohydrase) improved cellular immunity as indicated by the higher response or reaction to dinitrochlorobenzene and phytohemagglutinin after stimulation for 24 hours and the increased antibody titers toward Newcastle disease virus as well as the increased relative weight of lymphoid organs (spleen). Interesting finding was reported by Singh et al. (2019), in which Probiotic and multi-enzyme supplementation would be complementary to each other in nutritionally balanced feeds, and their combination would have a more pronounced influence on high-fiber diets than low-fiber diets. On this basis, the combination of probiotics and enzyme seems to be more beneficial when the poultry are fed low-quality diets.

Unlike the above-mentioned studies, a number of studies have documented the lack of synergistic effect between probiotics and enzyme on growth of poultry. Agboola et al. (2015a) reported no synergistic effect of probiotics (*Saccharomyces cerevisiae* and *Lactobacillus sporogenes*) and enzyme (carbohydrase) on the growth rate of broiler chickens compared to individual treatment with probiotic or enzyme alone. Likewise, Hosseini et al. (2017) did not find any synergistic effect of *Bacillus*-based probiotic and enzyme (carbohydrase) on gain of weight and feed efficiency of broiler strain. Wealleans et al. (2017) also reported that a combination of multi-strain *Bacillus* probiotic and multi-enzyme (xylanase, amylase and protease) could not further improve the growth performance and feed efficiency of broiler chickens when compared with the individual treatment using probiotic or multi-enzymes. Also, Kırkpınar et al. (2018) reported that the blends of probiotics (*Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* sub sp. thermophilus, *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum* subsp. *plantarum*, *Bifidobacterium bifidum*, *Candida pintoloppesii*, *Enterococcus*

faecium and *Asperigillus oryzae*) and commercial enzyme complex (protease, cellulose, amylase, xylanase, endo-1,3; 1,4-beta glucanase, lipase and -glucosidase, phytase) had no complementary impact on the growth rate of broilers. In contrast to the expectation, the combination of probiotics and enzyme exerted an antagonistic effect on the production of chickens in the study of Machado et al. (2020). They noticed that the combination of commercial probiotic (not specified) and xylanase resulted in lower weight gain than that of probiotic administration alone, and was not different from that of xylanase alone or control. The latter investigator also reported no significant different among the treatments with regards to carcass weight of broilers. Indeed the combination of commercial probiotic (not specified) and xylanase resulted in greater relative weight of abdominal fat pad as compared to probiotic or xylanase alone. Taken together, further investigation on using a combination of probiotics and enzymes needs to be explored especially with regard to which probiotics species/strains used as well as the types and doses of enzymes supplemented. In addition, the quality and compositions of feed/ration should be considered when applying the mixture of probiotics and enzymes as the dietary supplement, as it may affect the efficacy of additive as in-feed antibiotics alternative for broilers (Singh et al., 2019).

Combined Use of Probiotics and Organic Acids in Broiler Production

The synergistic activity of probiotics and acidifier-based organic acids against bacterial pathogens has been shown by an *in vitro* experiment. Bolivar et al. (2018) documented that the antibacterial activities of probiotic *Lactiplantibacillus plantarum* subsp*. plantarum* was more prominent when combined with organic acids (propionate, glutamate, succinate, butyrate, formate, fumarate, citrate and acetate) against *Aeromonas hydrophila*, *Vibrio alginolyticus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus agalactiae* compared to that of probiotics alone. It has been suggested that the drop in lactic acid bacterial pH (due to organic acid administration) may raise the contents of undissociated organic salts (toxic form), which in turn increases the bactericidal effectiveness of the probiotic bacteria (Sugiharto, 2016). Likewise, the application of organic acids, which is propionic acid, can be beneficial for the bacteria themselves as organic acid may create more favorable conditions for the lactic acid bacteria (Kung et al., 2004). In accordance with the above *in vitro* study, there has a recent trend in combining of probiotics and organic acid-based acidifiers to maximize the growth-and health-promoting effects of these feed additives on poultry (Sugiharto, 2016). Study in broiler chickens by Masud et al. (2016) confirmed that dietary administration of a combination of probiotics (*Bacillus licheniformis* and *Bacillus subtilis*) and organic acid (acetic acid, propionic acid, formic acid, lactic acidm sorbic acid and phosphoric acid) resulted in higher live weight and improved feed efficiency compared to the single application of probiotics or organic acids to broiler chickens. Compared to the individual use of the feed additive, the supplementation of blends of probiotics and acidifiers also improved the growth rate and nutrient use of broilers (Jadhao et al., 2019). Moreover, Saleem et al. (2018) confirmed that compared to single administration, the combined use of probiotics (hydrolyzed yeast mixture) and organic acid blend yielded in better growth and feed efficiency in broiler chickens and thus more beneficial. In line with modern broiler strains, a recent investigation on the Indonesian indigenous crossbred chickens showed that dietary inclusion of the mixture of *Bacillus subtilis* and butyric acid produced greater final body weight and weight gain relative to that of administrated with butyric acid alone or control (Widiastuti et al., 2019).

The latter investigators also found that carcass percentage (% live weight) was higher in the Indonesian indigenous crossbred chickens treated with the combination of *Bacillus subtilis* and butyric acid when compared with those of butyric acid alone or control. Other study by Sugiharto et al. (2019) also documented that live weight of the Indonesian indigenous crossbred chickens was greater when they were treated with the blend of *Saccharomyces cerevisiae* and formic acid when compared with those of treated with individual formic acid or *Saccharomyces cerevisiae* and control at 8 weeks of age. The improved feed conversion ratio was also observed in chicks provided with the blend of formic acid and *Saccharomyces cerevisiae*, relative to control and single administration of formic acid or *Saccharomyces cerevisiae* (Sugiharto et al., 2019). Although had no substantial impact on the growth rate of broiler, Rodjan et al. (2018) reported that feeding a mixture of probiotic and organic acid resulted in higher duodenal villi height and crypt depth than the birds fed on organic acid alone. This suggests that the blends of probiotic and organic acid were superior in improving the absorptive capacity of the chickens. The latter condition may ameliorate the nutrient utilization and thus growth of poultry. In accordance, El-Din et al. (2018) reported that a combined administration of commercial probiotic (Bioplus®*) and organic acid (citric acid salt) accounted to the higher ash retention and digestibility of fat and nitrogen free extract* in broiler chickens. The better feed intake in poultry received a combination of probiotic and organic acid may also be attributed to the better growth of poultry compared to

the single use of probiotic or organic acid (Widiastuti et al., 2019). It is also proposed that the synergistic antibacterial activity between probiotic and organic acid (Bolivar et al., 2018; Rodjan et al., 2018) may contribute to the better health status of poultry.

Different from the above reports, Gunal et al. (2006) showed no influence of the combined use of probiotics (the mixture of *Lactiplantibacillus plantarum* subsp*. plantarum, Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp*. bulgaricus, Lacticaseibacillus rhamnosus, Streptococcus thermophilus, Bifidobacterium bifidum, Aspergillus orizea, Enterococcus faecium* and *Candida pintolepesii*) and organic acids (blend of formic acid salts and propionic) on weight gain, feed consumption and feed efficiency relative to single use of probiotic or organic acids on broiler diets during the rearing of 42 days. Barbieri et al. (2015) also reported no significant effect of the mixture of *Bacillus amyloliquefaciens* and acetic, lactic and butyric acids on growth, intestinal histology and bacterial populations of broilers infected with *Eimeria* spp. Moreover, Dousa et al. (2016) did not find any difference among probiotic (*Bacillus subtilis*), organic acids (fumaric acid, citric acid, lactic acid, D-L malic acid and orthophosphoric acid) and the blend of probiotic and organic acid on growth, feed consumption and feed efficiency of broilers reared until day 42. Allahdo et al. (2018) showed no effect of combined use of probiotics (*Pediococcus acidilactici* MA 18/5M) and vinegar (containing 5% acetic acid) on the growth, immune and intestinal health of broilers, relative to treatment with probiotic or organic acid alone. The combined use of probiotics (*Pediococcus acidilactici*, *Limosilactobacillus reuteri*, *Enterococcus faecium* and *Bifidobacterium animalis*) and organic acids (formic acid) also showed no essential effect on weight gain and feed conversion of broilers challenged with *Campylobacter coli* in the investigation of Mortada et al. (2020). Jadhao et al. (2020) further pointed out that dietary administration of blends of probiotic (*Saccharomyces cerevisiae*) and organic acids (calcium propionate, sodium formate , fumaric acid, sorbic acid and citric acid) had no substantial impact on dressing percentage, giblet, edible meat percentage, boneless breast meat percentage and boneless leg meat percentage of broiler chickens. Elhassan et al. (2019) observe the absent synergistic or mutual support between probiotic *Bacillus subtilis* and organic acids (fumaric acid, citric acid, D-L malic acid, orthophosphoric acid and lactic acid) on gut morphology of broilers. Indeed, Agboola et al. (2015b) found that combining of probiotic (*Lactobacillus sporogenes* and *Saccharomyces cerevisiae*) and organic acids (ammonium formate, formic acid, ammonium propionate and propionic acid) resulted in compromised growth performance of broiler relative to administration of organic acid alone. Several explanations may be indicated for the inconsistent impact on the growth and health of poultry fed a mixtures of probiotic and organic acid (in comparison to the administration of probiotic or organic acid alone), including variations in species or forms, as well as doses of probiotic and organic acid, broiler breeds or strains, ration composition and experimental conditions. In respect particularly to the dose of organic acid used, El-Din et al. (2018) noticed the higher ash retention and nitrogen free extract digestibility in broiler supplemented with a combination of 0.05% commercial probiotic (Bioplus®*)* and 0.25% citric acid salt as compared to the mixture of 0.05% Bioplus® *and 0.5% citric acid salt.* The too acid condition in the gastrointestinal tract of broiler due to high amount of citric acid salt seems to exert acidic stress which may therefore compromise the nutrient digestibility and retention in broiler chickens (Nourmohammadi & Khosravinia, 2015).

Conclusions

Some reports show that the combined use of probiotics and other active ingredients resulted in synergistic or complementary effects, while other studies pointed out no mutual supportive effect between probiotics and its counterpart on broiler production. The variations in the species or strains of probiotic microorganisms, types and levels of the counterpart active compounds, quality and composition of feed provided to chicks, strains of broilers and experimental conditions may be accounted to the divergent effect between probiotics and other counterpart active ingredients on broiler production.

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References

Abdel-Hafeez, H. M., Saleh, E., Tawfeek, S. S., Youssef, I. & Abdel-Daim, A. (2017). Effects of probiotic, prebiotic, and synbiotic with and without feed restriction on performance, hematological indices and carcass characteristics of broiler chickens. *Asian-Australasian Journal of Animal Sciences, 30 (5)*, 672-682.

Abdel-Raheem, S. M., Abd-Allah, S. M. S., Hassanein, K. M. A. (2012). The effects of prebiotic, probiotic and synbiotic supplementation on intestinal microbial ecology and histomorphology of broiler chickens. *International Journal for Agro Veterinary and Medical Sciences, 6 (4)*, 277-289.

Agboola, A. F., Odu, O., Omidiwura, B. R. O. & Iyayi, E. A.

(2015a). Effect of probiotic, carbohydrase enzyme and their combination on the performance, histomorphology and gut microbiota in broilers fed wheat-based diets. *American Journal of Experimental Agriculture, 8 (5)*, 307-319.

Agboola, A. F., Omidiwura, B. R. O., Odu, O., Popoola, I. O. & Iyayi, E. A. (2015b). Effects of organic acid and probiotic on performance and gut morphology in broiler chickens. *South African Journal of Animal Science, 45 (5)*, 494-501.

Al-Baadani, H. H., Abudabos, A. M., Al-Mufarrej, S. I. & Alzawqari, M. (2016). Effects of dietary inclusion of probiotics, prebiotics and synbiotics on intestinal histological changes in challenged broiler chickens. *South African Journal of Animal Science, 46 (2)*, 157-165.

Alfaig, E., Angelovicova, M. & Kral, M. (2013). Effect of probiotics and thyme essential oil on carcass parameters of broiler chickens. *Scientific Papers: Animal Science and Biotechnologies, 46 (2)*, 50-52.

Ali, R. A. M., El-Shafey, A. S. & El-Kelawy, M. I. (2018). Immunophysiological and productive respose of broiler chicks to dietary supplementation with multi-enzyme and/or probiotics. *Egyptian Poultry Science Journal, 38 (4)*, 1047-1067.

Allahdo, P., Ghodraty, J., Zarghi, H., Saadatfar, Z., Kermanshahi, H. & Dovom, M. R. E. (2018). Effect of probiotic and vinegar on growth performance, meat yields, immune responses, and small intestine morphology of broiler chickens. *Italian Journal of Animal Science, 17 (3)*, 675-685.

Al-Sultan, S. I., Abdel-Raheem, S. M., El-Ghareeb, W. R. & Mohamed, M. H. A. (2016). *Japanese Journal of Veterinary Research, 64 (Suppl. 2),* S187-195.

Bai, S. P., Wu, A. M., Ding, X. M., Lei, Y., Bai, J., Zhang, K. Y. & Chio, J. S. (2013). Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. *Poultry Science, 92 (3)*, 663-670.

Barbieri, A., do Valle Polycarpo, G., Cardoso, R. G. A., da Silva, K. M., Dadalt, J. C., Madeira, A. M. B. N., de Sousa, R. L. M., de Albuquerque R. & Cruz-Polycarpo, V. C. (2015). Effect of probiotic and organic acids in an attempt to replace the antibiotics in diets of broiler chickens challenged with *Eimeria* spp. *International Journal of Poultry Science, 14 (11)*, 606-614.

Beski, S. S. M. & Al-Sardary, S. Y. T. (2015). Effects of dietary supplementation of probiotic and synbiotic on broiler chickens hematology and intestinal integrity. *International Journal of Poultry Science 14 (1)*, 31-36.

Blajman, J. E., Frizzo, L. S., Zbrun, M. V., Astesana, D. M., Fusari, M. L., Soto, L. P., Rosmini, M. R. & Signorini, M. L. (2014). Probiotics and broiler growth performance: a meta-analysis of randomised controlled trials. *British Poultry Science, 55(4)*, 483-494.

Bolivar, N. C., Legarda, E. C., Seiffert, W. Q., Andreatta, E. R. & do Nascimento Vieira, F. (2018). Combining a probiotic with organic salts presents synergistic *in vitro* inhibition against aquaculture bacterial pathogens. *Brazilian Archives of Biology and Technology, 61,* e18160694.

Çalik, A., Ekim, B., Bayraktaroğlu, A. G., Ergün, A. & Saçakli, P. (2017). Effects of dietary probiotic and synbiotic supplementation on broiler growth performance and intestinal histomorphology. *Ankara* Üniversitesi *Veteriner Fakültesi Dergisi, 64,* 183-189.

Das, O., Patil, S. S., Savsani, H. H., Padodara, R. J., Garg, D. D., Marandi, S. & Barad, N. (2016). Effect of dietary prebiotics, probiotics and synbiotics as feed additives on blood profile and broiler performance. *International Journal of Science, Environment and Technology, 5 (5)*, 3546-3552.

Dittoe, D. K., Ricke, S. C. & Kiess, A. S. (2018). Organic acids and potential for modifying the avian gastrointestinal tract and reducing pathogens and disease. *Frontiers in Veterinary Science, 5 (216)*.

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. *European Journal of Experimental Biology, 2 (6),* 2125-2129.

Dong, Z. L., Wang, Y. W., Song, D., Wang, W. W., Liu, K. B., Wang, L. & Li, A. K. (2019). Effects of microencapsulated probiotics and plant extract on antioxidant ability, immune status and caecal microflora in *Escherichia coli* K88-challenged broiler chickens. *Food and Agricultural Immunology, 30 (1)*, 1123-1134.

Dousa, B. M., Adam, M. S., Malik, H. E. E., Ali, O. H. A, Elhassan M. M. O, Elamin, K. M. & Elagib, H. A. A. (2016). Impact of probiotics and acidifiers on growth performance and blood chemistry of broiler chickens. *International Journal of Science and Research, 5 (10)*, 1670-1674.

Duskaev, G. K., Rakhmatullin, S. G., Kazachkova, N. M., Sheida, Y. V., Mikolaychik, I. N., Morozova, L. A. & Galiev, B. H. (2018). Effect of the combined action of *Quercus cortex* extract and probiotic substances on the immunity and productivity of broiler chickens. *Veterinary World*, *11 (10)*, 1416-1422.

El-Din, T. H. T., Awad, A. L. & Eid, O. A. M. (2018). Effect of dietary probiotic and citric acid addition on growth performance, carcass traits and blood parameters in broiler chicks. *Journal of Sustainable Agricultural Sciences, 44 (3)*, 123-133.

Elhassan, M. M. O., Ali, A. M., Blanch, A., Kehlet, A. B. & Madekurozwa, M.-C. (2019). Morphological responses of the small intestine of broiler chicks to dietary supplementation with a probiotic, acidifiers, and their combination. *Journal of Applied Poultry Research, 28 (1),* 108-117.

Elkhouly, M. A., Khairy, M. H., Abd-El Alim, A. F. & Ali, A. M. (2016). Effect of phytobiotics, probiotics and toltrazuril on chicken coccidiosis. *Zagazig Veterinary Journal, 44 (3)*, 214-223.

Ferdous, M. F., Arefin, M. S., Rahman, M. M., Ripon, M. M. R., Rashid, M. H., Sultana, M. R., Hossain, M. T., Ahammad, M. U. & Rafiq, K. (2019). Beneficial effects of probiotic and phytobiotic as growth promoter alternative to antibiotic for safe broiler production. *Journal of Advanced Veterinary and Animal Research, 6(3),* 409-415.

Flores, C. A., Duong, T., Askelson, T. E., Dersjant-Li, Y., Gibbs, K., Awati, A. & Lee, J. T. (2019). Effects of direct fed-microorganisms and enzyme blend co-administration on growth performance in broilers fed diets with or without antibiotics. *Journal of Applied Poultry Research, 28 (4)*, 1181-1188.

Ghasemi, H. A. & Taherpour, K. (2013). Comparative effects of probiotic, prebiotic and synbiotic supplements on performance, jejunal morphology, serum lipid profile and antibody response

of broiler chicks. *Journal of Livestock Science and Technologies, 1 (2),* 20-27.

Gheisar, M. M. & Kim, I. H. (2018). Phytobiotics in poultry and swine nutrition - a review. *Italian Journal of Animal Science, 17 (1)*, 92-99.

Gunal, M., Yayli, G., Kaya, O., Karahan, N. & Sulak, O. (2006). The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. *International Journal of Poultry Science, 5 (2),* 149-155.

Hosseini, S. M., Chamani, M., Mousavi, S. N., Hosseini, S. A. & Sadeghi, A. A. (2017). Effects of dietary physical form and dietary inclusion of probiotic and enzyme on growth performance, cellular and humoral immunity, and relative weights of lymphoid organs at early period of broiler chickens fed triticale-based diets. *South African Journal of Animal Science, 47 (6)*, 776-784.

Hussein, E. O. S., Ahmed, S. H., Abudabos, A. M., Aljumaah, M. R., Alkhlulaifi, M. M., Nassan, M. A., Suliman, G. M., Naiel, M. A. E. & Swelum, A. A. (2020a). Effect of antibiotic, phytobiotic and probiotic supplementation on growth, blood indices and intestine health in broiler chicks challenged with *Clostridium perfringens*. *Animals (Basel), 10 (3)*, 507.

Hussein, E. O. S., Ahmed, S. H., Abudabos, A. M., Suliman, G. M., Abd El-Hack, M. E., Swelum, A. A. & Alowaimer, A. N. (2020b). Ameliorative effects of antibiotic-, probiotic- and phytobiotic-supplemented diets on the performance, intestinal health, carcass traits, and meat quality of *Clostridium perfringens*-infected broilers. *Animals (Basel), 10 (4)*, 669.

Jadhao, G. M., Wankhede, S. M., Ali, S. S., Sawai, D. H., Kathale, N. J., Pawar, K. G., Gautam, M. J., Kolhe, R. P. & Rewatkar, H. N. (2020). Probiotic and acidifiers supplementation effect on carcass characteristics of broiler chicken. *Journal of Entomology and Zoology Studies, 8 (2)*, 1300-1303.

Jadhao, G. M., Wankhede, S. M., Rekhate, D. H., Dhok, A. P., Bankar, P. S. & Rewatkar, H. N. (2019). Effect of supplementation of acidifiers with probiotic on performance of broiler chicken. *Indian Journal of Animal Nutrition, 36 (3)*, 325-328.

Kamel, E. R. & Mohamed, L. S. (2016). Effect of dietary supplementation of probiotics, prebiotics, synbiotics, organic acids and enzymes on productive and economic efficiency of broiler chicks. *Alexandria Journal of Veterinary Sciences, 50 (1)*, 8-17.

Kırkpınar, F., Açıkgöz, Z., Mert, S. & Işık, Ö. (2018). Effects of dietary probiotic, prebiotic and enzyme mixture supplementation on performance, carcase, organ, ileal ph and viscosity of broilers. *Journal of Animal Production, 59 (2)*, 1-9.

Kung Jr, L., Myers, C., Neylon, J., Taylor, C., Lazartic, J., Mills, J. & Whiter, A. G. (2004). The effects of buffered propionic acid-based additives alone or combined with microbial inoculation on the fermentation of high moisture corn and whole-crop barley. *Journal of Dairy Science, 87 (5)*, 1310-1316.

Machado, N. J. B., Cruz, F. G. G., Brasil, R. J. M., Rufino, J. P. F., Freitas, L. W., Dilelis, F., Quaresma, D. V. & Lima, C. A. R. (2020). Effects of xylanase and probiotic supplementation on broiler chicken diets. *Revista Brasileira de Zootecnia, 49*, e20190216.

Markowiak, P. & Śliżewska**, K.** (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens, 10,* 21.

Masud, A. A., Ali, M. S. & Ahammad, M. U. (2016). Combined use of dietary probiotic and acidifier for the production of antibiotic free broiler. *Research in Agriculture, Livestock and Fisheries, 3 (1)*, 127-137.

Mohammed, L. S., Mohammed Abo-Salem, M. E., Atallah, S. T., El Shawarby, R. M. & Kamel, E. R. (2016). Effect of probiotics, prebiotics, synbiotics, organic acids and enzymes supplementation on broiler chicks' immunity in relation to the economic performance. *Benha Veterinary Medical Journal, 30 (2),* 34-44.

Momtazan, R., Moravej, H., Zaghari, M. & Taheri, H. R. (2011). A note on the effects of a combination of an enzyme complex and probiotic in the diet on performance of broiler chickens. *Irish Journal of Agricultural and Food Research, 50 (2)*, 249-254.

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 (2)*, 341-348.

Mortada, M., Cosby, D. E., Shanmugasundaram, R. & Selvaraj, R. K. (2020). In vivo and in vitro assessment of commercial probiotic and organic acid feed additives in broilers challenged with *Campylobacter coli*. *Journal of Applied Poultry Research, 29 (2)*, 435-446.

Murate, L. S., Paião, F. G., de Almeida, A. M., Berchieri Jr., A. & Shimokomaki, M. (2015). Efficacy of prebiotics, probiotics, and synbiotics on laying hens and broilers challenged with *Salmonella* Enteritidis. *Journal of Poultry Science, 52,* 52-56.

Nourmohammadi, R. & Khosravinia, H. (2015). Acidic stress caused by dietary administration of citric acid in broiler chickens. *Archives Animal Breeding, 58 (2)*, 309-315.

Olnood, C. G., Beski, S. S. M., Choct, M. & Iji, P. A. (2015). Novel probiotics: Their effects on growth performance, gut development, microbial community and activity of broiler chickens. *Animal Nutrition, 1 (3),* 184-191.

Prakasita, V. C., Asmara, W., Widyarini, S. & Wahyuni, A. E. T. H. (2019). Combinations of herbs and probiotics as an alternative growth promoter: An *in vitro* study. *Veterinary World*, *12 (4)*, 614-620.

Rahman, M. S., Mustari, A., Salauddin, M. & Rahman, M. M. (2013). Effects of probiotics and enzymes on growth performance and haematobiochemical parameters in broilers. *Journal of the Bangladesh Agricultural University, 11 (1),* 111-118.

Ren, H., Vahjen, W., Dadi, T., Saliu, E. M., Boroojeni, F. G. & Zentek, J. (2019). Synergistic effects of probiotics and phytobiotics on the intestinal microbiota in young broiler chicken. *Microorganisms, 7(12)*, 684.

Rodjan, P., Soisuwan, K., Thongprajukaew, K., Theapparat, Y., Khongthong, S., Jeenkeawpieam, J. & Salaeharae, T. (2018). Effect of organic acids or probiotics alone or in combination on growth performance, nutrient digestibility, enzyme activities, intestinal morphology and gut microflora in broiler chickens. *Journal of Animal Physiology and Animal Nutrition, 102 (2)*, e931-e940.

Saiyed, M. A., Joshi, R. S., Savaliya, F. P., Patel, A. B., Mishra, R. K. & Bhagora, N. J. (2015) Study on inclusion of probiotic, prebiotic and its combination in broiler diet and their effect on carcass characteristics and economics of commercial broilers. *Veterinary World, 8 (2),* 225-231.

Saleem, M. U., Masood, S., Zaneb, H., Durrani, A. Z., Aslam, A., Ashraf, K., Rashid, M. I., Habib-ur-Rehman, Muti-ur-Rehman, Ashraf, S., Usman, M. M. & Shaheen, M. S. (2018). Combined supplementation of organic acid blend and hydrolyzed yeast mixture increases growth performance and tibia bone strength in broilers. *Indian Journal of Animal Research, 52 (11),* 1613-1617.

Singh, A. K., Tiwari, U. P., Berrocoso, J. D., Dersjant-Li, Y., Awati, A. & Jha, R. (2019). Effects of a combination of xylanase, amylase and protease, and probiotics on major nutrients including amino acids and non-starch polysaccharides utilization in broilers fed different level of fibers. *Poultry Science, 98 (11)*, 5571-5581.

Sohail, M. U., Ijaz, A., Younus, M., Shabbir, M. Z., Kamran, Z., Ahmad, S., Anwar, H., Yousaf, M. S., Ashraf, K., Shahzad, A. H. and Rehman, H. (2013). Effect of supplementation of mannan oligosaccharide and probiotic on growth performance, relative weights of viscera, and population of selected intestinal bacteria in cyclic heat-stressed broilers. *Journal of Applied Poultry Research, 22 (3)*, 485-491.

Sugiharto, S. (2016). Role of nutraceuticals in gut health and growth performance of poultry. *Journal of the Saudi Society of Agricultural Sciences, 15 (2)*, 99-111.

Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., Wahyuni, H. I. & Sartono, T. A. (2019). Effect of formic acid, Saccharomyces cerevisiae or their combination on the growth performance and serum indices of the Indonesian indigenous crossbred chickens. *Annals of Agricultural Sciences, 64 (2)*, 206-210.

Tayeri, V., Seidavi, A., Asadpour, L. & Phillips, C. J. C. (2018). A comparison of the effects of antibiotics, probiotics, synbiotics and prebiotics on the performance and carcass characteristics of broilers. *Veterinary Research Communications, 42 (3)*, 195-207.

Wang, X., Farnell, Y. Z., Peebles, E. D., Kiess, A. S., Wamsley, K. G. & Zhai, W. (2016). Effects of prebiotics, probiotics, and their combination on growth performance, small intestine morphology, and resident *Lactobacillus* of male broilers. *Poultry Science, 95 (6),* 1332-1340.

Wealleans, A. L., Walsh, M. C., Romero, L. F. & Ravindran, V. (2017). Comparative effects of two multi-enzyme combinations and a *Bacillus* probiotic on growth performance, digestibility of energy and nutrients, disappearance of non-starch polysaccharides, and gut microflora in broiler chickens. *Poultry Science, 96(12)*, 4287-4297.

Widiastuti, E., Isroli, I., Murwani, R., Sartono, T. A., Wahyuni, H. I., Yudiarti, T. & Sugiharto, S. (2019). Dietary supplementation of butyric acid, probiotic *Bacillus subtilis* or their combination on weight gain, internal organ weight and carcass traits of the Indonesian indigenous crossbred chickens. *Livestock Research for Rural Development. 31 (9)*, http://www.lrrd.org/lrrd31/9/endwi31134.html

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