Effects of pre-, pro-, and synbiotic supplementation on the growth performance and feed conversion rates of Indonesian native chicken – The offspring of in ovo L-gln fed hen

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Abstract. A research was conducted to elucidate the effect of probiotic, prebiotic and synbiotic supplementation in drinking water on the performance of Indonesian Native Chickens (INC), the offspring of in ovo 1.5% L-gln hens. A randomized Block Design of 4 treatments (P0=control; P1=Prebiotic (10 g Inulin in 1 l tap water); P2= Probiotic (2 g Promix in 1 l tap water), and P3=Synbiotic (10 g Inulin + 2 g Promix in 1 l tap water) with 3 times of replication blocks was used. An amount of 180 one-day-old (DOC) of INC from 3 hatching periods was used in the research, which was selected from newly hatched chicks as the offsprings of laying hen resulted from the in ovo L-gln. The chicks were put in brooding boxes separately for each treatment unit (maximum 15 chicks per box) and placed in a rearing room (27-28°C; 60% RH). The results indicated that supplementation of pre-, pro-, and symbiotic during the first 2 weeks through drinking water did not significantly affect the body weight and feed conversion ratio (FCR) of INC. Afterward, measuring at weeks 4, 6, and 8, supplementation resulted in significantly heavier body weights and better FCR compared to those of control chicks. In conclusion, this study has shown that supplementation of pre-, pro-, and symbiotic exert positive effects on the growth and FCR of INC, and the best response is to symbiotic supplementation.

1. Introduction
There is growing interest in using a variety of pre- and probiotics to promote animal health and productivity, in broiler chicken particularly, because of increasing the bacterial resistance and decreasing acceptance of the farmer on using antibiotic - Antimicrobial Growth Promoters (AGPs). Some alternatives, referred to as Natural Growth Promoters (NGPs) have been identified as effective and safe alternatives to AGPs. At present, various kinds of NGPs are available in the market, including pro-, pre, and symbiotic (mixture of pro- and prebiotics).

Some studies have suggested that synbiotics are the best option to activate the metabolism of health-promoting bacteria and/or by selectively stimulating their host’s welfare and thus the growth [1-3]. The main importance of this form of synergism is that if provided a probiotic alone, without prebiotic as a source of nourishment, microorganisms in probiotics could not survive well in the digestive system. This synergism may apparently promote a better growth performance for the host.
Delivering bioactive substances, such as pro-, pre-, and symbiotic into the chicken’s gastrointestinal tract, can be conducted conventionally in-feed or in-drinking water supplementation, and delivering in-drinking water appears more superior than in-feed supplementation [4].

This study was to elucidate the effects of the commercial pro-, pre- and symbiotic supplementation through drinking water on performance (bodyweight) and feed conversion rate) of INC.

2. Material and method
A research was conducted to elucidate the efficacy of supplementation of Prebiotic, Probiotic, and its Symbiotic on INC growth performance of INC, which were the offsprings of in ovo fed laying hen. A Randomized Block Design of 4 supplementation treatment groups consisting of: P0 (control); P1=Prebiotic (10 g Inulin in 1 l tap water); P2 Probiotic = (2 g Promix in 1 l tap water) and P3=Symbiotic (10 g Inulin + 2 g Promix in 1 l tap water) with 3 times of replication blocks was used. Promix is a commercial probiotic contained ±6.2x10⁶ CPU per g, including Bacillus subtilis, Bifidobacterium bifidum, Bifidobacterium longum, Lactobacillus acidophilus, and Saccharomyces cerevisiae.

The research was conducted on 180 one-day-old INC (DOC) resulted from 3 hatching periods, which were selected from newly hatched chicks as the offsprings of the laying hen of in ovo L-gln fed. The chicks were weighed, fitted a wing tag, and placed in separate brooding boxes for each treatment unit (15 chicks per box), and supplementation conducted up to 4 weeks of age and placed in a room of Animal Physiology Laboratory, then continued in the animal house up to 8 weeks of age.

The chicks were fed with the commercial ration containing 21.0 g protein /kg, and 13.0 MJ metabolizable energy (ME)/kg. Food, tap water and treated water were provided ad libitum, and monitored daily, while body weights were measured 2 weekly up to 8 weeks of age.

Statistical analysis was performed using the statistical software package SPSS for Windows (version 20.0; SPSS Inc., Chicago, IL, USA). Statistical significance between mean values was set.

3. Result and discussion
The results of the research are presented in tabel 1 and tabel 2. The initial body weights among the treatment groups of selected newly hatched chicks of INC were not significantly different.

There were no significant effects of supplementation up to 2 weeks of age; The positive responses of the chicks on supplementation of pro-, pre-, and symbiotic appear after 2 weeks which is measured at 4 weeks of age, which then continues at 6 and 8 weeks of age. At 4, 6 and 8 weeks of age, the chicks supplemented with the symbiotic had greater (P<0.05) body weight compared to those of control chicks. Moreover, pre- and probiotic supplemented chicks had a greater body weight than those of control chicks.

Feed conversion ratio (FCR) was lower for the supplemented chicks compared to the control chicks. This response was indicated after two weeks of age. Interestingly, that FCRs of chicks supplemented pro-, pre-, and symbiotic at 4 week age were significantly lower compared to those at the other ages including the control chicks.

Growth performance, including body weight and feed conversion, is the general and direct indicator in poultry production as it involves feed utilization and overall effectiveness of poultry production. The main role of the diet is not only to provide adequate nutrition to meet the metabolic needs of the body but also to regulate various body functions. The responses of body weights and FCRs are of course due to the effects of pro, pre, or symbiotic supplementation.

**Table 1. Effects of Pre-, Pro-, and Synbiotic Supplemented in drinking water on body weight of INC.**

| Age (week) | P0       | P1       | P2       | P3       | Sig      |
|------------|----------|----------|----------|----------|----------|
| Newly HW (g) | 31.05±3.03 | 31.25±3.69 | 31.08±3.13 | 31.10±3.16 | P>0.05   |
| 2          | 77.33±5.63 | 79.67±7.43 | 80.00±6.45 | 79.58±6.56 | P>0.05   |
| 4          | 206.67±8.51 | 219.33±13.21 | 238.33±14.67 | 245.67±11.48 | P<0.05   |
| 6          | 315.00±24.78 | 329.33±20.96 | 342.20±28.20 | 345.40±21.69 | P<0.05   |
| 8          | 492.07±22.88 | 507.13±23.86 | 514.00±33.70 | 540.47±26.69 | P<0.05   |

Mean within the same row with different superscripts are significantly different (P<0.05)
The effects of probiotics on performance parameters of INC, body weight and FCR, are in agreement with the results of previous studies on broiler chickens that fed probiotics which resulted in improvement in growth performance and feed efficiency [5-9]. This response is thought to be induced by the effects of probiotic action including the maintenance of beneficial microbial population, improving feed intake and digestion [10], and altering bacterial metabolism [11,12]. Moreover, some characteristic effects of the immune system, decrease inflammatory reactions, prevent pathogen proliferation, reducing ammonia and urea production and excretion, and enhance animal performance. These characteristics have been investigated and mostly conducted on commercial breeds of chicken, either on broiler chicken [13,14] or laying hen [15-17].

Prebiotics interact selectively with the host to stimulate favourable microbiota in the digestive ecosystem and may have valuable effects in reducing the incidence of enteric pathogens [18]. Prebiotics has a synergistic effect with probiotics, and a systemic effect on the utilization of feed ingredient by the host, stimulation of immunity, and neutralization of toxins and they exert their action by lowering pH through lactic acid production and thus inhibiting the colonization of pathogenic bacteria [19-21].

There are three crucial elements in the gut lumen of the chicken as an ecosystem: (1) microbial community, (2) intestinal epithelial cells, and (3) immune system [22]. Prebiotic can be fermented by health-promoting bacteria (probiotic supplemented) in the intestine, producing lactic acid, short-chain fatty acid (SCFA), and some antibacterial substances, such as bacteriocin against pathogenic species [23]. These substances may not only beneficial for the intestinal microbial structure but also improve the integrity of intestinal epithelial cells, which further increase the absorption of nutrients and enhance the growth performance of chicken [24], as seen in the results of the present study reported herein.

The synergism between probiotic and prebiotic called the symbiotic would have greater benefits than using probiotic or prebiotic alone, because in the combination, the prebiotic may enhance the growth and activity of the used probiotic species [25], without prebiotic as a source of nourishment, microorganism in probiotic could not survive well in the digestive system. In the present study, INC treated with symbiotic (P3) which is a mixture between probiotic (P1) and prebiotic (P2) resulted in a significantly heavier body weight (P<0.05) and a significantly better feed conversion rate (P<0.05) compared to those of control (P0), and this performance is indicated after week 2.

Moreover, it appears that the FCR of the chicks in P1, P2, and P3 measured at week 4 were better than those measured at weeks 6 and 8, including control chicks. Some possibilities may be attributable with these results, and intriguing for further study. Firstly, functional maturity of the digestive system of INC may apparently be achieved after week 2, in spite of supplementation of pro, pre-, and symbiotic had been started soon after hatching. Secondly, the efficacy of duration, dosages, and brand or kind of supplementation via drinking water require evaluation. In the present study, supplementation was conducted up to week 4, used commercial pro-, pre-, and symbiotic with the dosages as suggested in the label.

### 4. Conclusion

In conclusion, the results of this study indicate that supplementation of Prebiotic and Probiotic, either alone or together as Symbiotic were responded positively by INC, which are indicated by heavier body weight and lower FCR than those of the control. These responses appeared after 2 weeks of supplementation.
supplementation - measured at weeks 4, 6, and 8. An indication that symbiotic supplementation resulted in a better response of INC compared to pre-, and probiotic.

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References
[1] Gibson G R and Roberfroid M B 1995 Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics J. Nutr. 125 1401–12
[2] Sławińska A, Siwek M, Żylińska J, Barmdowski J, Brzezińska J, Guliewicz K A, Nowak M, Urbanowski M, Płowiec, A, Bednarczyk M 2014 Influence of synbiotics delivered in ovo on immune organs development and structure Folia Biol. 62 277–85
[3] Kamel E R and 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 J. Vet. Sci. 50 8–17
[4] Torshizi M A K, Moghaddam A R, Rahimi S and Mojgani N 2010 Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response Br. Poult. Sci. 51 178–84
[5] Cavazzoni V, Adami A and Castrovilli C 1998 Performance of broiler chickens supplemented with Bacillus coagulans as probiotic Br. Poult. Sci. 39 526–29
[6] Zulkifli I, Abdullah N, Azrin N M and Ho Y W 2000 Growth performance and immune response of two commercial broiler strains fed diets containing Lactobacillus cultures and oxytetracycline under heat stress conditions Br. Poult. Sci. 41 593-97
[7] Kabir SML 2009 The role of probiotics in the poultry industry Int. J. Mol. Sci. 10 3531–46
[8] Mountzouris K C, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G and Fegeros K 2007 Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities Poult. Sci. 86 309–17
[9] Abdel-Hafeez H M, Saleh E S, Tawfeek S S, Youssef I M and Abdel-Daim A S 2017 Effects of probiotic, prebiotic, and synbiotic with and without feed restriction on performance, hematological indices and carcass characteristics of broiler chickens. Asian-Australas. J. Anim. Sci. 30 672-82
[10] Khana S H, Yousaf S, Mian A A, Rehmana A and Farooq M S 2011 Assessing the effect of administering different probiotics in drinking water supplement on broiler performance, blood biochemistry and immune response J. Appl. Anim. Res. 39 418-28
[11] Smirnov A, Perez R, Amit-Romach E, Sklan D and Uni Z 2005 Mucin dynamics and microbial populations in chicken small intestine are changed by dietary probiotic and antibiotic growth promoter supplementation J. Nutr. 135 187–192
[12] Pourabedin M and Zhao X 2015 Prebiotics and gut microbiota in chickens FEMS Microbiology Letters 362 15
[13] Al-Sultan S I, Abdel-Raheem S M, El-Ghareeb W R and Mohamed M H 2016 Comparative effects of using prebiotic, probiotic, synbiotic and acidifier on growth performance, intestinal microbiology and histomorphology of broiler chicks Japanese J. Vet. Res. 64 S187-195
[14] Al-Khalifa H, Al-Nasser A, Al-Surayee T, Al-Kandari S, Al-Enzi N, Al-Sharrah T, Ragheb G, Al-Qalab S and Mohammed A 2019 Effect of dietary probiotics and prebiotics on the performance of broiler chickens Poult. Sci. 98 4465-79
[15] Youssef A W, Hassan H M A and Ali H M 2013 Effect of probiotics, prebiotics and organic acids on layer performance and egg quality Asian J. Poult. Sci. 7 65-74
[16] Xiang Q, Wang C, Zhang H, Lai W, Wei H and Peng J 2019 Effects of different probiotics on
laying performance, egg quality, oxidative status, and gut health in laying hens Animals 9 1110-19
[17] Peralta-Sánchez J M, Martín-Platero A M, Ariza-Romero J J, Rabelo-Ruiz M, Zurita-Gonzáles M J, Banana A, Rodríguez-Ruano S M, Maqueda M, Valdivia E and Martínez-Bueno M 2019 Egg production in poultry farming is improved by probiotic bacteria Front. Microbiol. 10 1042-55
[18] Alloui M N, Szczurek W and Swiatkiewicz S 2013 The usefulness of prebiotics and probiotics in modern poultry nutrition: A review Ann. Anim. Sci. 13 17–32
[19] Patterson J A and Burkholder K M 2003 Application of prebiotics and probiotics in poultry production Poult. Sci. 82 627–31
[20] Hajati H and Rezaei M 2010 The application of prebiotics in poultry production Int. J. Poult. Sci. 9 298-304
[21] Teng P Y and Kim W K 2018 Roles of prebiotics in intestinal ecosystem of broilers Front. Vet. Sci. 5 245
[22] Lavelle E C, Murphy C, O’Neill L A and Creagh E M 2010 The role of TLRs, NLRs, and RLRs in mucosal innate immunity and homeostasis Mucosal Immunol. 3 17–28
[23] Boguslawska-Tryk M, Piotrowska A and Burlkowska K 2012 Dietary fructans and their potential beneficial influence on health and performance parameters in broiler chickens J. Centr. Eur. Agric. 13 270–88
[24] Lan Y, Verstegen M, Tamminga S and Williams B 2005 The role of the commensal gut microbial community in broiler chickens World’s Poult. Sci. J. 61 95–104
[25] Bozkurt M, Aysul N, Kucukyilmaz K, Aypak S, Ege G, Catli A U, Aksit H, Coven F, Seyrek K and Cinar 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 Poult. Sci. 93 389–99