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The effect of combination *Bifidobacterium sp* and *Lactobacillus acidophilus* probiotic on egg yolk cholesterol, HDL, and LDL

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Abstract. The purpose of this study to evaluate the effects of combination 0.5% *Bifidobacterium sp.*+0.5% *Lactobacillus acidophilus* on egg yolk cholesterol, High density lipoprotein (HDL) and low density lipoprotein (LDL) in hens. Methods: this research using completely randomized design. Sixty layers were randomized into three treatments, each treatment contains four replicates and each replicates contains five hens. Twenty five week-old Isa Brown layers were fed without probiotic (T0), 0.1% antibiotic growth promoters (T1), 0.5% *Bifidobacterium sp.*+0.5% *L. acidophilus* (T2) for 30 days. Result: the result showed that there was a significant difference between the control and all treatment groups on egg yolk cholesterol, HDL and LDL. Supplementation of 0.5% *Bifidobacterium sp.*+0.5% *L. acidophilus* probiotic decreased the egg yolk cholesterol and LDL ($p<0.05$) and increased HDL in egg yolk ($p<0.05$) levels. The lowest egg yolk cholesterol and LDL by supplementation 0.5% *Bifidobacterium sp.*+0.5% *L. acidophilus* probiotic (155.86 and 48.46 mg/dL) respectively, compared the control (224.0200 and 58.6300 mg/dL) and the highest egg yolk HDL by supplementation of 0.5% *Bifidobacterium sp.*+0.5% *L. acidophilus* probiotic (50.00 mg/dL) compared the control treatment (41.5900 mg/dL). The conclusion of this study was the supplementation of 0.5% *Bifidobacterium sp.*+0.5% *L. acidophilus* could improve egg yolk quality of hens.

1. Introduction

Probiotics or direct-fed microbials are live microorganisms which beneficially affect the host animal by improving their intestinal microflora [1] and they were living symbiotically in the digestive tract of poultry which is known to increase host immunity through control of pathogenic bacteria [2] by improving their intestinal microflora [3]. The supplementation of probiotics has been found could increase the egg production, egg weight, and feed efficiency in laying hens [4, 5, 6]. Ishihara reported

that an increase in the amount of *Lactobacillus* spp in laying hens is beneficial in preventing pathogenic diseases [7].

The characterize of probiotic has adhesive properties and can colonize different parts of the avian digestive tract [8]. Lactic acid bacteria, such as *Pediococcus acidilactici*, *Lactobacillus* spp., *Lactococcus lactis* can produce lactic acid from the results of glucose metabolism, which serves to acidify the digestive environment, and bactericide effect that can inhibit pathogenic bacteria growth such as *E. coli*, *Salmonella Typhimurium* [9]. Manipulation of intestinal microbial with probiotics plays an important role in the lipid metabolism of chickens.

The many studies have shown that probiotics could reduce cholesterol level in egg yolk [10, 11] and reduce cholesterol level in serum, carcass and liver of broiler chickens [8, 12, 13]. The study by Ramasamy showed that *Lactobacillus* culture in the feeding of hens can decrease the cholesterol level of eggs at 24 and 28 at 32 weeks of age [14]. Kimoto has been reported that a culture of *Lactobacillus acidophilus* taking up cholesterol actively by in vivo to exert a hypocholesterolemic effect [15].

It was known that some strains of *Bifidobacteria*, *Lactobacillus fermentum* KC5b, *Lactobacillus acidophilus johnsonii* and *Lactobacillus delbrueckii* JCM 1002 were able to remove cholesterol from culture medium during anaerobic condition growth in the presence of bile acid enrichment, and it depends on strains [16]. This research is important to prove the ability of lactic acid bacteria *Bifidobacterium sp* and *Lactobacillus acidophilus* to decrease cholesterol, LDL and increase HDL in the egg production. The objective of this recent study was to evaluate the effect of the combination probiotics contain *Bifidobacterium sp* and *Lactobacillus acidophilus* could affect on cholesterol, LDL and HDL in the egg yolk of laying hens.

2. Materials and methods

This research is quantitative. A total of 60 laying hens (Isa Brown) at 23 weeks of age was randomized under standard managed conditions. Each laying hen was placed into each battery cage (20x35x35 cm) of an open house system. The cages were assigned at random among 3 treatments, with 4 replicates, each with 5 laying hens; the treatments were as follows: 1) control, basal diet without probiotics and without AGPs; 2) basal diet with 0.1% AGPs; 3) basal diet with 0.5% *Bifidobacterium sp* + 0.5% *Lactobacillus acidophilus*. Basal diets and water were provided ad libitum. At the end of 4-week period, eggs were selected at random from each treatment and the yolks separated. Duplicate sub-samples were taken and then analyzed for total cholesterol, LDL and HDL. The quantitative value of cholesterol, LDL and HDL were determined by using UV-Vis spectrophotometer methods.

All data treatment was analyzed using the analysis of variance procedure and treatment mean differences were tested using the least significant difference. The level of significance of the analyses was set at $p < 0.05$. The computation was done using the SPSS program 21 version.

3. Results and discussion

The effects of probiotics on the HDL level of egg yolk by using UV-Vis spectrophotometer presented in Table 1. The results of a recent study using probiotics showed that there was a significant difference ($p < 0.05$) between the treatment of egg yolk HDL. The highest HDL level (50.00 mg/dL) was found in the treatment using 0.5% *Bifidobacterium sp* + 0.5% *Lactobacillus acidophilus* and the lowest egg yolk cholesterol level was found in the control without probiotics and without AGPs (41.59).

Table 1. Average of high density lipoprotein (HDL) of egg yolk in the treatment.

Treatment	Average HDL (mg/dL)
P0=control	41.59 ^a
P1=0.1% AGPs	43.36 ^b
P2= 0.5% <i>Bifidobacterium sp</i> + 0.5% <i>Lactobacillus acidophilus</i>	50.00 ^c

The effects of probiotics on the LDL level of egg yolk by using UV-Vis spectrophotometer presented in Table 2. The results of a recent study using probiotics showed that there was a significant difference ($p < 0.05$) between the treatment of egg yolk LDL. The highest LDL level was found in the control without probiotics and without AGPs (58.63 mg/dL), while the lowest egg yolk cholesterol level was found in the treatment using 0.5% *Bifidobacterium sp* + 0.5% *Lactobacillus acidophilus* (48.460 mg/dL).

Table 2. Average of low density lipoprotein (LDL) of egg yolk in the treatment.

Treatment	Average LDL (mg/dL)
P1=0.1% AGPs	48.463 ^a
P2= 0.5% <i>Bifidobacterium sp</i> + 0.5% <i>Lactobacillus acidophilus</i>	48.460 ^a
P0=control	58.63 ^b

The effects of probiotics on cholesterol level of egg yolk by using UV-Vis spectrophotometer presented in Table 3. The effects of supplementation with *Bifidobacterium sp* and *Lactobacillus acidophilus* probiotic on egg yolk in laying hens showed there were significant differences between the treatment. The egg yolk cholesterol level was decreased ($p < 0.05$) compared the control and AGPs treatment. The highest cholesterol level was found in the control without probiotics and without AGPs (224.02 mg/dL), while the lowest egg yolk cholesterol level was found in the treatment using 0.5% *Bifidobacterium sp* + 0.5% *Lactobacillus acidophilus* (155.86 mg/dL).

Table 3. Average of cholesterol of egg yolk in the treatment.

Treatment	Average cholesterol (mg/dL)
P2= 0.5% <i>Bifidobacterium sp</i> + 0.5% <i>Lactobacillus acidophilus</i>	155.86 ^a
P1=0.1% AGP	211.41 ^b
P0=control	224.02 ^c

This recent study in line with the other research that showed significant results in decrease of egg yolk cholesterol content by used the 0.1% Lactic Acid Bacteria, contain *Lactobacillus paracasei* (KKP 824), *Lactobacillus rhamnosus* (KKP 825) and *Lactobacillus rhamnosus* (KKP 826) (17) with concentration 6.7×10^8 cfu/g [17]. The other research showed that the addition of probiotic had a significant effect on decrease egg yolk cholesterol (mg/g of yolk) [4]. The study by Haddadin showed that the giving of probiotic *Lactobacillus acidophilus* can decrease the egg cholesterol level in 40 weeks of production [10]. The recent study also in line with the study by Abdulrahim, the result showed that the effect of *Lactobacillus acidophilus* was a significant decrease in the cholesterol level in the egg yolks (16.95%) and serum (56.24%). Giving *Lactobacillus acidophilus* after 12 weeks of culture administration can reduce cholesterol level in the blood, through process deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis [18].

The use of *Bifidobacteria sp* in this recent study in line with a Liong's research show that *Bifidobacteria* strains could remove of cholesterol from the nutrient medium incorporated with cholesterol. The cholesterol removal was associated with growth of organisms. It was determined by the difference in cholesterol level in the medium after and before of the incubation period. Several mechanisms have been proposed to reduce cholesterol that included absorption cholesterol by growing cells of the intestine [16], cholesterol binding to cell surface [19, 20], incorporation into cellular membrane [15], deposition of cholesterol by conjugated bile salts [21].

The decreased absorption of cholesterol in the intestinal through co-precipitation of intestinal cholesterol with the deconjugated bile salt, which is observed at pH values below 5.5 [16]. However, some of these mechanisms depend on the strain bacteria and laboratory conditions [20]. It was

reported that fed broilers by probiotic (*Lactobacillus acidophilus*, *Bifidobacteria*, *Aspergillus oryzae*) have lower levels of cholesterol compared with control group [22]. The decrease of cholesterol level by probiotic bacteria is due to the modulation of lipid metabolism, incorporation and assimilation of cholesterol in the cell membrane of the probiotics, intestinal conversion of cholesterol in coprostanol, and inhibition of the expression of the intestinal cholesterol transporter Niemann–Pick C1 like 1 (NPC1L1) in the enterocytes [23].

4. Conclusion

Based on the result, it could conclude that the supplementation of a combination 0.5% *Bifidobacterium sp* + 0.5% *Lactobacillus acidophilus* probiotic could improve egg yolk quality of laying hens by decreased cholesterol and LDL and increase HDL in egg yolk.

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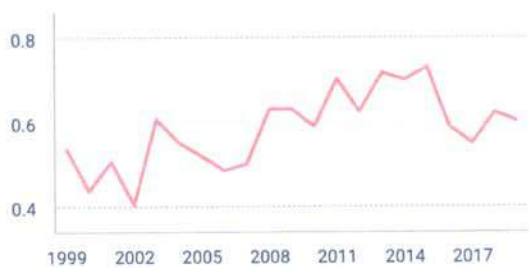
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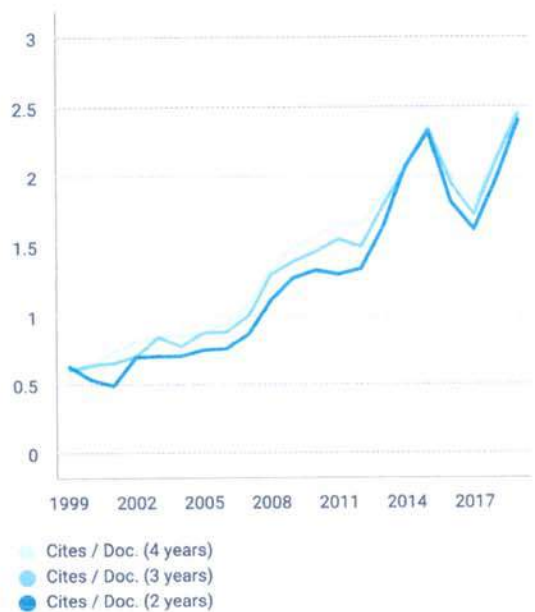
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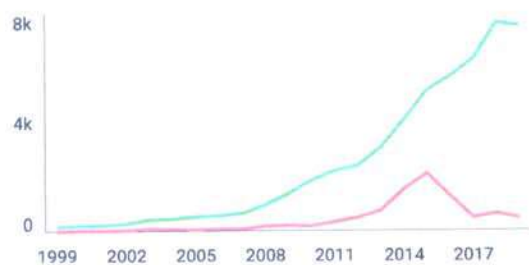
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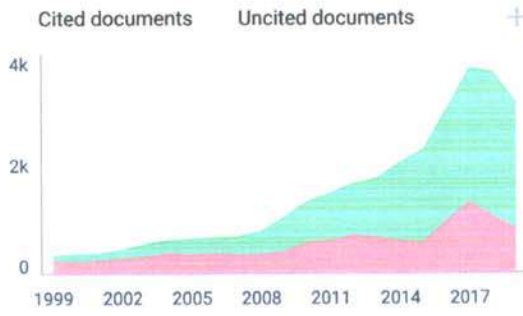
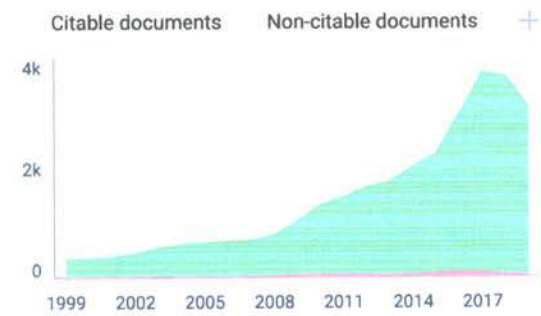
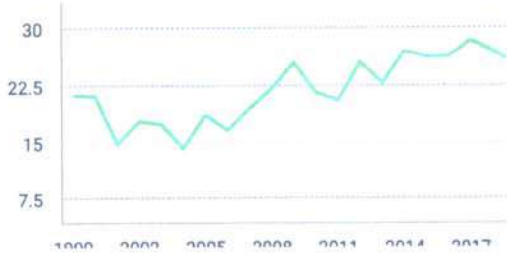
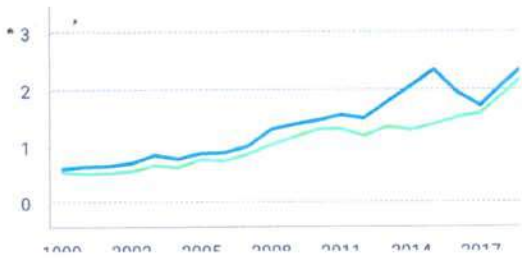
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