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Original Article

Effect of Liquid Probiotics Mixed Culture Supplements through Drinking Water on Laying Hens Performance and Yolk Cholesterol

Sesotya Raka Pambuka*, Osfar Sjofjan and Lilik Eka Radiati

Department of Feed and Animal Nutrition, University of Brawijaya, Malang, Indonesia
Corresponding author's email: rakaart06@gmail.com; 116050117011008@mail.ub.ac.id

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ABSTRACT

The effects of Liquid Probiotic Mixed Culture (LPMC) on laying hens performance were studied. One hundred twenty eight 44-wk-old Isa Brown layers were randomly divided into 2 groups with 64 laying hens or layers in two groups. Layers in first group were fed with commercial feed as antibiotic contained diets; the other fed with self-mixed feed as antibiotic free diets. Both of groups had different LPMC level: control, 0.15 %, 0.30 % and 0.45 % of LPMC (v/v) added in water fount. The method used in this research was completely randomized design and followed by Duncan's multiple tests. The results showed better improvements on antibiotic free diets in decreasing yolk cholesterol (P<0.01). LPMC supplementation did not improve performances either on antibiotic free and commercial diets. Increased level of LPMC supplementation did not give significant effect on feed and water consumption, hen day production, egg weight, feed conversion, whereas overall variables had improvement tendency numerically and commercial feed groups had better performances than antibiotic free diets. LPMC supplementation did not improve layers performance but could be substitute antibiotics.

Key words: Liquid Probiotic Mixed Culture, Antibiotic, Laying Hens Performance, Yolk Cholesterol

INTRODUCTION

Over a decade ago a significant proportion of the European poultry industry removed in-feed Antibiotic Growth Promoters (AGPs) following pressure from the major markets due to concerns regarding possible antibiotic resistance (Ratcliff, 2000). Indonesian poultry industry also concerned it, thus researcher attempt to subtitute antibiotics use but until now the efforts have serious problems, one of them was difficulty on application and haven't significant effect and an ignorance about antibiotics impact (Kompiang, 2004). Over the years the word probiotic has been used in several different ways to replacing probiotic. Since Metcnikoff's work with beneficial bacteria in sour milk called *bulgarian bacillus* (Fuller, 1992). The use of probiotics on current science has developed. Fuller (1989) later gave a unique definition of probiotics as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.

Probiotics have several beneficial impact, including stimulating appetite (Nahashon et al., 1992; Nahashon et al., 1993), improve intestinal microbial balance (Fuller, 1989), stimulate the immune system (Toms and Powrie, 2001), produce the digestive enzyme (Gilliland and Kim, 1984; Saarela et al., 2000), utilize undigestible carbohydrate (Prins, 1977), produce toxic compounds such as volatile fatty acids and release bacteriocins (Rolfe, 2000) that compete with other microbes for adhesive site (Dunham *et al*., 1993). Reduce meat and yolk cholesterol by secretion bile salt hydrolase (Begley *et al*., 2006). Regarding the controversial results about using biological additives, the strain, concentration and form of them (viability, dryness or their products) should be considered (Mahdavi *et al*, 2005). Feeding viable *Lactobacillus* at 1100 mg/kg (4.4 $\times 10^7$ cfu/mg) increased daily feed consumption, egg size, nitrogen and calcium retentions and decreased intestinal length from 7 to 59 weeks of age (Nahashon et al., 1996). Haddadin *et al.* (1996) reported that egg production, egg size and egg quality were improved by the addition of a liquid culture of *Lactobacillus acidophilus* to the basal diet. Sjofjan (2003) reported yolk cholesterol by the addition of *Bacillus* spp. lower than control that using AGPs. Xu *et al*. (2006) also reported layers when added by 500 mg of *B. subtilis* culture/kg improve egg production, feed consumption, feed conversion and increase eggshell thickness, yolk color, Haugh unit and decreases in yolk cholesterol concentration.

Liquid Probiotics Mixed Culture (LPMC) contains two type microorganisms, *Lactobacillus* sp. and *Bacillus* spp. Premise of the combinations was to escalate beneficial impacts on host either on health

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status and performances. Probiotics microorganism is scarce living individually. It happens more often symbiotic with other especially endogenous microorganisms (Siliker, 1980; Margino and Rahayu, 1992; and Panek, 1993). Indonesia is a developing country. It have many small farmers than huge farmers, which prefer traditional raising method, low input cost and expect high output income. Hence, commercial feed not always a primary option diets, farmers preferred self-mixed feed because give high benefit (Sjofjan, 2003). The objective of this study was to investigate the effect of probiotic mixed culture supplements through drinking on laying hen's performance and egg cholesterol and the comparison on self-mixing feed and commercial feed.

MATERIALS AND METHODS

Animals

One hundred twenty eight 44-wk-old Isa Brown layers were used. Layers were raised in bamboos double deck stairs cage containing 4 layers with 1-wk for pre-experimental test and 8-wk for LPMC trial on different feed groups and supplementation level. Each cage was provided with plastic feeder and water fount.

Cultures Used

LPMC contained two different strain probiotic microorganisms (*Lactobacillus* sp. and *Bacillus* spp.). LPMC was collected from Petrokimia Kayaku Co. Indonesia, and it contained approximately $1x10^9$ viable cells/ml.

Treatments

The commercial feed was from Ali Farm, East Java, Indonesia and antibiotic free feed was self-mixed. The composition and nutrient analysis of the diets showed in Table 1.

Two fed groups and four levels LPMC supplementation, with 4 layers each treatment, were carried out. Layers in treatment 1 were fed commercial diets (A1) with different LPMC supplementation levels on water fount. Those are control, 0.15 % v/v, 0.30 % v/v, 0.45 % v/v. Layers in treatment 2 were fed an antibiotic free diet (A2) with different LPMC supplementation levels on water fount. Those are control, 0.15 % v/v, 0.30 % v/v, 0.45 % v/v. The experimental period for all treatments was 60 days. Feed was restricted as much as 120 g/layer/day and water drinking was restricted as much as 300 ml/layer/day.

Sampled feed were collected monthly and analyzed for ME, CP, CL, CF, Calcium and phosphorus contents of the experimental diets were calculated, whereas methionine and lysine calculated in the end of research. Hen Day Production (HDP) was recorded for 7 day to check for similar pre-experiment values for treatments. The water consumption and egg weight were determined daily. The feed consumption and Feed Conversion Ratios (FCR) were determined at 7 day intervals. Water and feed consumption, HDP, egg weight, and FCR using formula described by Scott *et al*. (1992). The yolk cholesterol was determined monthly and analysis by Subekti (2006) method.

Tabel 1. Composition and Nutrient analysis

Composition	**Unit**	**A1 Feed** *	**A2 Feed** **
Corn	%	50,51	56,72
Rice bran	%	15,78	17
Concentrate	%	31,57	-
Mineral	%	1,89	1,89
Premix	%	0,25	0,25
Meat Bone Meal	%	-	3
Poultry Meat Meal	%	-	6
Soybean Meal	%	-	15
DL-methionine	%	-	0,14
Total		100	100
Nutrient analysis ***			
Metabolizable Energy (ME)	(Kkal/kg)	2805,23	2741,37
Crude Protein (CP)	(%)	18,06	17,87
Crude Lipid (CL)	(%)	5,13	4,41
Crude Fiber (CF)	(%)	6,07	9,65
Ca	(%)	3.87	2.84
P	(%)	0.28	0.64
Methionine	(%)	0.31	0.43
Lysin	(%)	0.77	0.90

Source: *Ali Farm formulation, East Java, Indonesia. **Self-mixed feed formulation. ***Analysis result from Feed Nutrition Laboratory, Brawijaya University, Indonesia.

Statistical Analysis

Data were analyzed with ANOVA (Steel and Torrie, 1992). The research design used completely randomized design. Differences among treatment were tested using Duncan's multiple comparison test and statistical significance was declared at P<0.05 and P<0.01.

RESULTS

This study had evaluated the effects of LPMC on water and feed consumption, HDP, egg weight and FCR. Table 2 showed the effects of LPMC in feed groups on layer hens performances. Table 3 show the effects of LPMC level on layer hen's performances.

Feed group 1(A1) was commercial feed as antibiotic contained diets has the highest result on feed consumption was found on 0.45 % (v/v) LPMC supplementation (119.96±0.06) and lowest on 0.15 % (v/v) LPMC supplementation (119.79±0.12); water consumption had the highest result on control (279.56±23.53) and lowest on 0.15 % (v/v) LPMC supplementation (262.10±2.91); HDP has the highest result on 0.45 % (v/v) LPMC supplementation (92.41±3.46) and lowest on control (90.63±5.75); egg weight had the highest result on 0.45 % (v/v) LPMC supplementation (68.12±2.04) and lowest on control (65.22±1.59); FCR had highest result on control (2.05±0.12) and lowest on 0.30 % (v/v) LPMC supplementation (1.92±0.10); yolk cholesterol had the highest result on 0.30 % (v/v) LPMC supplementation (216.88±0.64) and lowest on 0.45 % (v/v) LPMC supplementation (216.11±0.29).

Feed group 2 (A2) was self-mixed feed as antibiotic free diets has the highest result on feed consumption was found on 0.15 % (v/v) LPMC supplementation (118.00±0.92) and lowest on 0.45 %

(v/v) LPMC supplementation (115.25±2.28); water consumption has the highest result on 0.30 % (v/v) LPMC supplementation (117.76±1.90) and lowest on 0.45 % (v/v) LPMC supplementation (253.98±9.98); HDP had the highest result on 0.45 % (v/v) LPMC supplementation (76.34±3.05) and lowest on control (73.55±4.00); egg weight had the highest result on 0.30 % (v/v) LPMC supplementation (63.77±0.90) and lowest on control (63.12±1.46); FCR has the highest result on 0.45 % (v/v) LPMC supplementation (2.78± 0.29) and lowest on control (2.48±0.11); yolk cholesterol had the highest result on control (214.75±0.91) and lowest on 0.45 % (v/v) LPMC supplementation (213.19±0.45). In addition, statistical analysis result on Feed and water consumption, HDP, egg weight and FCR was no significantly different on consumption ($P>0.05$), but yolk cholesterol was significantly different ($P<0.01$). These results was in line with Kompiang (2004) report, that indicated the addition of either GPA, *Bacillus apriarius* or commercial probiotic did not affect significantly on HDP, egg weight and FCR.

Table 2. Effect of LPMC supplementation in feed groups on layer hens performances (52-wk-old)

Parameter	Unit	A1	A2
Feed consumption	g/layer/day	119.90±0.08	116.72±1.37
Water consumption	ml/layer/day	274.34±8.23	269.64±16.69
HDP	%	91.32±0.76	74.78±1.39
Egg Weight	g/egg	66.66±1.37	63.12±0.58
FCR		1.98±0.06	2.65±1.37
Yolk cholesterol	mg/100mg	216.49±0.42	213.82±0.68

Table 3. Effect of LPMC supplementation level on layer hens performances (52-wk-old)

Feed Groups	Level	Parameter					
		Feed consumption*	Water consumption*	HDP*	Egg Weight*	FCR*	Cholesterol **
		g/layer/day	ml/layer/day	(%)	(g/egg)		(mg/100mg)
A1	(0 %) control	119.91±0.04	279.56±23.53	90.63±5.75	65.22±1.59	2.05±0.12	216.13±1.15
	(0.15 %) L1	119.79±0.12	262.10±2.91	91.07±1.82	65.81±1.81	2.01±0.05	216.83±0.83
	(0.30 %) L2	119.94±0.01	276.96±6.08	91.18±3.31	67.50±1.39	1.92±0.10	216.88±0.64
	(0.45 %) L3	119.96±0.06	278.75±16.31	92.41±3.46	68.12±2.04	1.95±0.02	216.11±0.29
A2	(0 %) control	115.85±1.96	259.15±7.01	73.55±4.00	63.12±1.46	2.48± 0.11	214.75 ± 0.91^{b}
	(0.15 %) L1	118.00±0.92	274.43±22.74	73.66±3.84	63.23±1.19	2.70± 0.19	213.85 ± 0.27^{ab}
	(0.30 %) L2	117.76±1.90	291.02±38.08	75.56±5.21	63.77±0.90	2.66± 0.22	213.50 ± 0.59^{a}
	(0.45 %) L3	115.25±2.28	253.98±9.98	76.34±3.05	62.35±1.79	2.78± 0.29	213.19 ± 0.45^{a}

Attn: Means ± SEM with different superscript are very significantly different (**) ($P<0.01$) and significantly different (*) ($P<0.05$).

DISCUSSION

Lower feed consumption on self-mixed feed primary caused by heterogenic particle size, while on commercial feed was homogenous. Rizal (2006) reported feed consumption affected by physic look of feed, Layers prefer crumble form rather than mash form of feed and nutrient content was instrumental on feed consumption. LPMC supplementation did not improve the variables on self-mixed feed, even the feed was antibiotic free diets. The results was inconsistent with Sellars (1991) report, probiotic addition could be improves nutrient availability in intestine and recover nutrient intestinal absorption, so that give effectively decrease feed consumption because layers have enough nutrient for maintenance and production.

During the 8-wk period feeding trial, the comparison within commercial feed as antibiotic contained diets and self-mixed feed as antibiotic free diets was showed on table 2. Best results on feed consumption (119.90±0.08), water consumption (274.34±8.23), HDP (91.32±0.76), egg weight (66.66±1.37), FCR (1.98±0.06) was found in the group consuming commercial feed than self-mixed feed, whereas yolk cholesterol (213.82± 0.68) was better in the group consuming self-mixed feed than commercial feed.

Probiotic addition did not improve egg weight significantly, which has already been reported by Mohan *et al*. (1995) and Haddadin *et al*. (1996). Complementary reports by the Nahashon et al. (1996) and Haddadin *et al*. (1996) suggested that addition of biological additives did not influence the egg weight significantly ($P>0.05$). These results might be related to the dosages of probiotic and concentration of bacteria used in the diet. FCR had not significant difference. Our findings are consistent with those from Nahashon et al. (1994). However, there are also conflicting research results concerning the effect of dietary probiotic supplementation. Leeson and Major (1990) suggested that only under stressful conditions do coliforms increase in numbers and probiotics have measurable benefit.

The result showed that comparison between feed consumption and water consumption was approximately 1: 2.30. It could be indicating increased 1 g feed consumption resulting in increased 2.30 ml water consumption. These results corroborate an earlier study by Ensminger *et al*. (1990) indicated that commonly layers consume water 2 times greater than the amount of feed consumed as drinking water. Water serves as a solvent and transport nutrients in layers to spread around the body so that it takes more water than food. HDP, egg weight and FCR closely related with feed intake and feed nutrient, this means that the

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nutrient content of self-mixed feed are inadequate enough to forming an egg.

Addition of probiotic had significant effect on egg yolk cholesterol (mg/100mg of yolk). Haddadin *et al*. (1996) observed a similar response. They reported that inclusion of lactobacillus acidophilus in three ages (40, 44 and 48 week) affects egg cholesterol in 40 week of production.

CONCLUSIONS

- In conclusion, the addition of liquid probiotic mixed cultures gives a better effect on the layers fed with self-mixed feed than commercial feed in lowering yolk cholesterol.
- HDP, egg weight, and FCR better on commercial feed because of the feed consumption were lower in layers fed with self-mixed feed and liquid probiotic mixed cultures did not affect water consumption.
- Higher levels liquid probiotic mixed cultures addition trough drinking water will further lowering yolk cholesterol. However, no significant effect on feed and water consumption, HDP, egg weight and FCR.
- Probiotics supplementation has not shown the optimum point in this research. Therefore, further research should focus on appropriate technology in probiotic manufacturing to improve the quality of probiotic and investigating gastrointestinal flora and mucosal cellular and humoral immunities when using probiotic as a substitute on improving business efficiency in small poultry farm.

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