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Dietary supplementation of powdered and encapsulated probiotic: *In vivo* study on relative carcass, giblet weight and intestinal morphometry of local duck

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ABSTRACT. This research was aimed to evaluate the effect of dietary supplementation of either powdered or encapsulated probiotic on relative carcass, giblet weight and intestinal morphometry of local duck. One hundred twenty male day old duck (DOD) were distributed to 6 different dietary groups, included 2 probiotic forms of either powdered (T1) or encapsulated (T2) and 3 levels: 0% (L0), 0.2% (L1), 0.4% (L2). They were reared using pen cages for 42 days (6 weeks). Observed variables were relative carcass, giblet weight (gizzard, heart, liver) and intestinal morphometry (villus height, villus width, crypt depth). Data were analyzed by Nested of Completely Randomized Design ANOVA and if there was significant effect followed by Duncan's Multiple Range Test (DMRT). The result showed that there was no significant effect ($p > 0.05$) of the form of either powdered or encapsulated probiotic on relative carcass, giblet weight, and intestinal morphometry. However, increasing level of probiotic have significant effect ($p < 0.05$) on relative carcass, villus height, and villus width, but did not significantly affect giblet weight and crypt depth. In conclusion, supplementation of either powdered or encapsulated probiotic has similar result, but it is suggested to use 0.4% of encapsulated probiotic (4 kg ton^{-1} of feed) in local duck diet.

Keywords: feed; poultry; nutrition; probiotic.

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Introduction

Local duck serves animal protein source of human. Nowadays, food safety and healthy from livestock products are the success key for sustainable livestock production system. Dietary synthetic feed additive has been used as an effective means to improve productivity, but it was banned by Indonesian government regulation through Permentan No. 14/2017. This ban due to pathogenic contamination, bacterial resistant in the end product and an effort to create sustainable poultry farming. Moreover, the use of antibiotics causes residual veterinary drugs in an animal product like meat, milk, and eggs (Jeong, Kang, Lim, Kang, & Sung, 2010).

Probiotics proposed as an alternative natural growth promoter due to probiotic is non-pathogenic live-microorganism which gives many health benefits to the host. Several studies have shown that the use of probiotics in the feed could improve the performances, population of micro flora, white blood cells, cholesterol content (Bansal & Bilaspuri, 2011; Natsir, Sjofjan, Widodo, Ardiansah, & Widyastuti, 2019). Using synthetic materials, such as butylated hydroxyanisole and butylated hydroxytoluene, which have been widely used in feed, was prohibited due to safety reason. Recent studies reported that using alter materials from bio-resources such as probiotic, prebiotic, phytobiotic were suggested. Antioxidant properties from *Lactobacillus casei* spp. (i.e. L. casei 114001) and *Lactobacillus fermentum* ME-3improved the ability to suppress oxidation of luminol, microsomal lipid peroxidation (consecutively was 43.0-65.8%, 57.9-89.5) and decreased of antioxidant capacity of blood plasma, liver and small intestines of animals elevated while malondialdehyde (MDA) content in blood plasma. The cultured-supernatant, intact cells, and intracellular cell-free extracts of Bifidobacterium animals were found to scavenge hydroxyl radicals and superoxide anion *in vitro* while enhancing the anti-oxidase activities of mice as *in vivo* treatment (Shen et al, 2011).

Antioxidant which expressed by probiotics were reported can improve production performance of broiler. Dietary supplementation of Lactobacillus acidophilus D2/ CSL (CEST 4529) were reported increase body weight at 28 d, daily weight gain and decrease feed conversion ratio also daily feed intake of broiler (De Cesare et al., 2017).

Protecting probiotic from unsupported environmental condition in the inside of gastrointestinal tract of broiler was important to be observed. Because, not all of probiotic that given to broiler would be adsorbed by target organ (small intestines), this was caused the barrier from pH condition inside of GIT, acidic-bile, enzyme, and another environmental condition that reduced amount of probiotics before entering to the target organ. After that, it has reduced viability and quantity-loss of probiotics (Corona-Hernandez et al., 2013; Gbassi & Vandamme, 2012).

Encapsulation method is potentially method that can improve the viability and survivability of broiler. This method coated the probiotic to promote immune function and improve growth performances of the broiler. In this research, we use either without encapsulation or encapsulation technology to protect probiotics. Because encapsulation technology has been observed to be a protector for core-material (either pro or prebiotic), this is an effective way to maintain viability, survivability and protect from environmental barriers inside, against pathogenic bacteria, enhance immune function and growth performances (Natsir et al., 2019; Ritzi, Abdelrahman, Mohnl, & Dalloul, 2014; Yazhini, Visha, Selvaraj, Vasanthakumar, & Chandran, 2018; Zhang et al., 2015). Supplementation of either powdered or encapsulated probiotic is expected to improve production performances. In this research, we would like to observe it then performed by relative carcass, giblet weight measurements and morphometric assay on the small intestine of local duck.

Material and methods

Local and time

This research was conducted within 2 months, during March-April 2017. Preparation for probiotic, either powder or encapsulated were conducted in Laboratory of Animal Feed and Nutrition, Faculty of Animal Science, University of Brawijaya. Testing TPC (Total Plate Count) was conducted in Laboratory of Microbiology, Faculty of Animal Science, University of Brawijaya. The *in vivo* test was conducted in Sumbersekar's Field Laboratory, Faculty of Animal Science, University of Brawijaya which located in Sumbersekar Village, Dau Sub-district, Malang Regency. The temperature ranged for 20-26°C and its humidity ranged for 60-80%.

Birds

Birds used in this research were a hundred twenty local male day old duck (DOD). The average weight of DOD was 37,81 ± 7,03%. The DOD was obtained from Karangploso, Batu City.

Feed

The basal diet used consisted of corn, rice bran, meat bone meal, soybean meal, salt, grit, DL-methionine, lysine, salt, palm oil, and premix. The result of proximate analysis of feed content is shown in Table 1.

Probiotic

In this research, we used multistrain of powdered-probiotic, consist of *Lactobacillus fermentum, Lacobacillus acidophilus, Bacilus spp*) that was obtained from commercial probiotics from Animal Nutrition and Feed Laboratory, Faculty of Animal Science, University of Brawijaya. TPC (Total Plate Count) testing was performed, and the viability obtained from this probiotic powder was >10^6 cfu g^{-1}. The process of making probiotic powder using Sjofjan (2014). The encapsulated probiotic procedure is using the method (Natsir, Osfar, Ilham, & Siti, 2018). Materials which used were probiotics, gum arabic, whey protein, and BHT (Butylated Hydroxy Toluene). The process of preparing the probiotic encapsulation, including mixing of 75% gum arabic and 25% whey and 0.06% BHT. After that, liquid probiotics prepared through the fermenter tube and carried out optimization for 1 hour at 40°C. Then mixing with encapsulant and mix for 3 hours at 37-40°C.

Table 1. Composition of the basal feed.

Feedstuff	(%)	
	(0-2 weeks)	(3-6 weeks)
Corn	56.2	61.5
Rice bran	12.3	12.5
Soybean meal	16.83	12.8
Corn Gluten Meal	8.14	6.4
Grit	1.2	1.4
Salt	0.1	0.2
DL-methionine	0.54	0.3
Lysine	0.36	0.4
Dicalcium phosphate	0.2	0.2
Premix	0.43	0.5
Palm Oil	3.7	3.8
Total	100	100
Nutrient Content*		
Metabolizable Energy (kcal kg^{-1})	3,100	3,100
Crude Protein (%)	20.60	18.60
Crude Fat (%)	4.95	4.95
Ca (%)	2.01	2.01
P Available (%)	0.40	0.40
Lysine (%)	0.88	0.88
Methionine (%)	0.43	0.43

*Proximate analysis was assayed by Laboratory of Animal Feed and Nutrition, Faculty of Animal Science, Universitas Brawijaya. Premix used was Topmix ™ (containing (per 10 kg): Vitamin A 12,000,000 IU; Vitamin D3 2,000,000IU; Vitamin E 8,000IU; Vitamin K 2,000 mg; Vitamin B1 2,000 mg; Vitamin B2 5,000 mg; Vitamin B6 500 mg; Vitamin B12 12,000 g; Vitamin C 25,000 mg; Calcium-D-panthothenate 6,000 mg; Niacin 40,000 mg; Choline chloride 10,000 mg; Methionine 30,000 mg; Lysine 30,000 mg; Manganese 120,000 mg; Iron 20,000 mg; Iodine 200 mg; Zinc 100,000 mg; Cobalt 200 mg; Copper 4,000; mg Zinc Bacitracin 21,000 mg); Palm Oil used was Sania ™ (containing: Total energy 70 kkal; Vitamin A 118RE; Vitamin E 8 mg).

Housing

The cage used in this research is a cage of opened house; the research cages were 70 cm x 70 cm x 70 cm by 30 units each and separated. Then, the brooding cage used 2.5 m x 2.5 m to 3.5 m - 3.5 m. Each unit is equipped with a 20 watts incandescent lamp which is used as a heater and lighting at the grower phase

Experimental Design

Method which used in this research was Nested of Completely Randomized Design (CRD) with two main factors, either form or level concentration of probiotic. Forms which used were powder (T1) and encapsulated (T2). Meanwhile, level concentration (L) which used were 0%, 0.2% and 0.4%. Each treatment consist of 5 replications, each replication consist of 4 birds. Treatment is given while the ages of duck were 2 weeks-6 weeks. Feed and drink were given *ad libitum*. The experimental method showed below:

T1L0: basal feed + 0% powder probiotic T1L1: basal feed + 0.2% powder probiotic T1L2: basal feed + 0.4% powder probiotic T2L0: basal feed + 0% encapsulated probiotic T2L1: basal feed + 0.2% encapsulated probiotic T2L2: basal feed + 0.4% encapsulated probiotic

Variables

The variables observed in this research were relative carcass, giblet weight (gizzard, heart, and liver), intestinal morphometry (length villi, width villi, and depth crypth) in small part of ileum. The relative carcass (%) was determined by carcass as a percentage of live body weight (Falaki, Shams, Dastar, & Zrehdaran, 2010). The giblet weight was measured according to Yadav, Meenu, Maousami, and Karnam (2018). The morphometric measurements were performed using a microscope (Olympus) at an objective magnification of 4 times with the help of a video microscope (Video measuring gauge IV-560, for Company Limited) at 5 fields for each preparation.

Data analysis

Data were analyzed by Analysis of Variance (Anova) Nested of Completely Randomized Design (CRD). When significant effect appeared followed by Duncan's Multiple Range Test (DMRT).

Results and discussion

In this experiment, using form and level model (powdered and encapsulated) of probiotic and its response to relative carcass, giblet weight and intestinal morphometry showed on Table 2. and Table 3.

Table 2. Effect of supplementation either powdered or encapsulated probiotic on relative carcass, giblet weight, and intestinal morphometry (form comparison).

Variables	Treatments		SEM	p-value
	Powdered	Encapsulated		
Relative carcass (%)	60.21±1.7	61.19±1.4	1.49	0.21
Gizzard weight (g 100 g^{-1})	3.97±0.29	3.57±0.21	0.24	0.15
Heart weight (g 100 g^{-1})	0.59±0.10	0.58±0.07	0.08	0.08
Liver weight (g 100 g^{-1})	3.59±0.22	3.57±0.34	0.31	0.17
Villus height (μm)	497.33±31.7	514.52±19.2	24.22	0.13
Villus width (μm)	119.41±4.1	122.11±5.8	4.54	0.27
Crypt depth (μm)	74.72±5.4	76.30±3.2	3.39	0.28

Table 3. Effect of supplementation either powdered or encapsulated probiotic on relative carcass, giblet weight, and intestinal morphometry (level comparison).

Variables	Level of powdered probiotic in the diet (%)			SEM	P-value
	0	0.2	0.4		
Relative carcass (%)	59.08±1.9[a]	60.16±2.3[a]	61.40±0.8[b]	1.87	<0.05
Gizzard weight (g 100 g^{-1})	3.976±0.21	3.944±0.43	3.990±0.23	0.33	0.16
Heart weight (g 100 g^{-1})	0.572±0.04	0.660±0.11	0.550±0.14	0.08	0.07
Liver weight (g 100 g^{-1})	3.420±0.19	3.788±0.16	3.570±0.30	0.29	0.08
Villus Height (μm)	489.7±41.35[a]	490.52±26.83[a]	511.78±26.25[b]	31.42	<0.05
Villus Width (μm)	118.71±4.99[a]	119.1±5.31[a]	120.428±1.68[a]	3.22	<0.05
Crypt Depth (μm)	74.01±5.88	74.65±3.93	75.52±7.24	4.90	0.12
	Level of encapsulated probiotic in the diet (%)				
Relative carcass (%)	59.08±1.9[a]	61.86±1.0[b]	62.64±1.3[b]	1.39	<0.05
Gizzard weight (g 100 g^{-1})	3.976±0.21	3.942±0.25	4.012±0.18	0.18	0.08
Heart weight (g 100 g^{-1})	0.572±0.04	0.606±0.70	0.588±0.10	0.07	0.09
Liver weight (g 100 g^{-1})	3.420±0.19	3.602±0.51	3.704±0.30	0.41	0.07
Villus Height (μm)	498.10±32.30[a]	522.21±12.88[b]	523.26±9.27[b]	23.67	<0.05
Villus Width (μm)	116.56±4.44[a]	123.07±4.63[b]	126.7±3.51[c]	4.49	<0.05
Crypt Depth (μm)	75.27±3.09	76.67±4.30	76.96±2.41	2.04	0.11

The effect of probiotic supplementation either powdered or encapsulated (form) on relative carcass

The effect of either powdered or encapsulated probiotic on relative carcass of local ducks were summarized in Table 2 and Table 3. The result of relative carcass showed that there was no significant effect ($p > 0.05$). While, level of either powder or encapsulated (Table 3) probiotics on relative carcass revealed that the treatments had significant ($p < 0.05$). Several studies explained that the relative carcass increased by increasing the protein content of basal feed. Supplementation probiotic bacteria could be enhanced the availability of protein. Similarly nutrient content such as metabolic energy, crude fiber and crude protein in the basal diet of the treatment seemed to be responsible for the divergent results. The feed containing higher crude fiber decreased carcass weight than feed with a lower crude fiber. The differences in quality of feed and growth rate seemed to contribute to the growth performance. In the previous study (Sarangi et al., 2016) reported that supplementation pre-probiotic had no significant effect on carcass of broilers. Furthermore, a non significant effect was found during supplementation Bacillus subtilis on carcass traits (Yadav et al., 2018). In collaborated with prebiotic on laying hen, Natsir et al. (2018) also reported that no effect on laying hen performances. Alkhalf, Alhaj, and Al-Homidan (2010) reported that higher level of the supplementation probiotic to broilers in the levels of 0.8 – 1 g kg^{-1} was found to be better than control. Increasing levels of encapsulated probiotic could improve viability and stability, increase protection against pathogenic resulting in improving in nutrient utilization, gut environment, and growth performance (Natsir et al., 2018; Zhang et al., 2015).

The effect of probiotic supplementation either powdered or encapsulated (form) on giblet weight

The effects of either powder or encapsulated probiotic on gizzard weight of local ducks were summarized in Table 2. Statistical analysis of gizzard and heart weight showed there was no significant ($p > 0.05$)

difference between experimental groups. This indicated that inclusion of probiotic and their combination did not stimulate the internal organ weight (Parsa et al., 2018). This finding was in line with (Shabani, Nosrati, Javandel, & Kioumarsi, 2012) that different level of probiotics had no statistically effect on gizzard weight. Similarly, Midilli et al. (2008) reported was no significant impact on gizzard relative weight during of probiotics and mannan-oligosaccharides in broiler chicks. A non-significant impact was observed by (Karaoglu & Durdag, 2005) that dietary probiotic *Saccharomyces cerevisiae* had no significant effect on gizzard weight, while the age of slaughter affected to the gizzard relative weight compared to control group. The factors affect gizzard weight are breed, genetics, age, sex, individual status and feed intake. Moreover, feeding poultry more than the requirement cause extra-digestion and it would affect the size of gizzard due to the thickening of the muscle, type of feed particle size (mash, crumble or pellet) affected giblet weights, smaller particle size decrease gizzard activity (Tuli, Sandhu, Kashyap, & Sharma, 2014). There was no significant difference observed by Sarangi et al. (2016) that dietary supplementation prebiotic, probiotic and symbiotic had no significant effect on liver weight of Cobb broilers. In contrast, Seifi et al. (2017) reported that addition pre-probiotics in diets comprising rice brand was increase the relative heart weight compared to the control (with no pre-probiotic). Increasing heart weight also found during supplemented *Bacillus subtilis* and *Enterococcus faecium* to broilers compared to the control group. Meanwhile, heart enlargement in broiler was affected by heat stress (Hatab, Elsayed, & Ibrahim, 2016; Khan et al., 2016). The divergent result might be influenced by strain, method of probiotic preparation, level of treatment, and basal composition (Zhang, Zhou, Ao, & Kim, 2012).

Supplementation of either powder or encapsulated probiotic on liver weight summarized in Table 2. The result demonstrated no significant ($p>0.05$) difference among the different experimental groups. Increasing level of probiotic did not significantly affect to liver weight due to the probiotic non-toxic or antinutrition-free. A presence of toxic in the basal feed increase in metabolic rate and causes abnormalities of organ weight as a result of reduce toxic effect. Feeding 0.5 and 0.8 ppm aflatoxin (AFB1) in broiler chicken was significant increase the relative weight of heart during period of 42 days (Lakkawar, Narayanaswamy, & Satyanarayana, 2017). Accumulation of lipids in the hepatocytes would change the heart size due to the severe fatty change, lipidosis, and inflammatory reaction (Lakkawar et al., 2015). This result in agreement with Awad, Ghareeb, Abdel-Raheem, and Böhm (2009) explained that supplementation probiotic had no significant difference in the weight of liver as compared to the control group. Increasing beneficial bacteria such as Lactic acid probiotic that decrease the activity of acetyl-CoA could reduce the lipid synthesis (Zhou et al., 2009).

The effect of probiotic supplementation either powdered or encapsulated (form) on intestinal morphometry

The effect of either powder or encapsulated probiotic forms on villus height (μm), villus width (μm) and crypt depth (μm) showed in Table 2. The effect of the use of either powdered or encapsulated probiotic showed that either powdered or encapsulated probiotic has no significant effect ($p > 0.05$) on the morphometry of the small intestine (villus height, villus width, and the crypt depth). There was appropriate because either powdered or encapsulated probiotic has the same ability to repair the local duck's gut microvilli. This research was supported by (Matur & Eraslan, 2012) which reported that growth of microvilli affected by several factors such as age, sex, and type of animal. Moreover, feed nutrients, environmental conditions of the cage, humidity, temperature and natural factors such as weather and seasons. A study reported by Maiorka et al. (2016) said that difference villus height due to age phase, it also was reported that the height age villi 60 wks longer than the age of 30 wks.

The effect of either powdered or encapsulated probiotic (level) on relative carcass

The effect of level of either powder or encapsulated (Table 3) probiotics on relative carcass showed there was significant effect ($p < 0.05$). This may due to the level of addition improve body weight and greater carcass weight obtained. As increasing the levels of probiotic used increased the relative carcass due to the presence of probiotics in the feed could improve the digestibility of nutrients by producing *bacteriocin* as an antibacterial that altering the growth of pathogenic bacteria. In the gastrointestinal tract, probiotic would break down proteins and carbohydrates into amino acids, N, and the soluble carbon that require synthesizing of proteins. Increased protein digestibility affects the improvement of protein metabolism, thus directly increasing the protein synthesis of meat.

The effect of either powder or encapsulated probiotic (level) on giblet weight

The effect of levels of either powder or encapsulated (Table 3) probiotics on giblet weight showed there was no significant difference (p > 0.05). Although increasing levels used tended to increase gizzard, heart, and weight liver relatively. This may due to the addition of different levels amount of different probiotic forms ranged between 0-0.4% did not significantly affect the weight of giblet. Moreover, indicated that the same nutrient content in the feed of dietary such as protein and fat content, age of slaughter, and anti-nutrition. Mc-lelland (1990) said that factors affected were size, color, and consistency of liver such as breed, genetics, age, sex, individual status and feed intake. Sarangi et al. (2016) reported that liver weight and gizzard weight in Cobb broilers under their study had no significantly different, then were agreed with Sahin, Kaya, Unal, and Elmali (2008) and Saiyed et al. (2015) that there was no effect using mixed probiotic and prebiotic in carcass quality of quail and broiler.

The effect of either powder encapsulated probiotic (level) on intestinal morphometry:

The effect of level of either powder or encapsulated (Table 3) probiotics on the villus height and villus width showed were significant effect (p < 0.05), but did not significantly affect (p > 0.05) to the crypt depth. There was assumed that the level of feed supplemented contains more probiotics, thus optimizing the growth of the villi due to probiotics are more susceptible to deterioration of viabilities before entry into intestinal microvilli. In addition, the villus width is also determined by the basal feed content consumed. This is in contrast to research conducted Harimurti of probiotic supplementation could increase villi width. Dong et al. (2016) reported that disruption of GIT epithelial cells causes micro- encapsulation of *Enterococcus faecium* does not work on the crypt. Another reason have reported that several aspects such as increased mRNA expression regulation of MUC2, then induced mucus protein secretion.

Conclusion

Supplementation of either powdered or encapsulated probiotic resulted similar result on relative carcass, giblet weight and intestinal morphometry. Using 0.2% and 0.4% level of encapsulated probiotic on relative carcass, villus height and villus weight better than powdered probiotic

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