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Effect of yeast and multi vitamin-minerals mix in diet to stimulate the growth performance of ducks and suppress pathogenic bacteria

Eny Puspani[1]*, Desak Putu Mas Ari Candrawati[1], and I Gusti Nyoman Gde Bidura[1]
[1]Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia
*Email: eny_fapet@unud.ac.id; bidura_unud@yahoo.com

ABSTRACT

The research aims to examine the effect of a mixture probiotic yeast *Saccharomyces spp.* and multi-vitamin-mineral complex (Provinal) to stimulate the growth of ducks and bio-control pathogenic bacteria in the intestine of ducks. Two hundred and forty one-day-old male ducks (*Anas sp.*) were used in a completely randomized design experiment allocated into 4 treatments, namely: P0) rations without Provinal; ration with the addition of 0.1%; 0.2%; and 0.30% Provinal, as treatment P1; P2; and P3, respectively. All treatment diets were in the form of mash containing 16% protein and 2900 kcal/kg metabolized energy. This study showed that administration of 0.10-0.30% Provinal increased average daily gains, feed efficiency, and feed digestibility ($P<0.05$). The concentration of cholesterol in serum, fecal N-NH3, the number of Choliform and E.coli bacteria in the intestines in treatments P1, P2, and P3, were lower ($P<0.05$) compared to P0. Conclusion that 0.10-0.30% Provinal supplemented in rations of ducks were increased daily weught gains, feed efficiencies, and nutrient digestibility but decreased serum cholesterol, $N\text{-}NH_3$ in excreta, total population of *Choliform* bacteria and *E. coli* in ducks.

Key words
Probiotic, ammonia, Choliform, E.coli, digestibility, ducks

Introduction

The most important thing in duck farming is to improve the health of ducks and nutrient intake to get maximum growth. Since 2018, the Indonesian government has banned the use of antibiotics in poultry feed, so it is necessary to find an alternative to the use of antibiotics in feed. Interesting to study is the use of yeast mixtures as a source of probiotics and multi-vitamin-minerals in feed to increase the growth of ducks and bio-control pathogenic bacteria.

This research is a way/step to find the best strategy in stimulating the growth of ducks as germplasm, as well as bio-control against pathogenic bacteria, so that feed efficiency and the problem of prohibiting the use of antibiotics in animal feed can be resolved.

Probiotic microbes are expected to synergize with the microbes in the duck intestines, so that it will have a beneficial effect through increasing the absorption and digestibility of feed [1,2]. Provision of probiotic cultures for poultry will be able to provide a beneficial effect between probiotic microbial species and digestive tract microbes of ducks, thereby increasing the feed digestibility [2,3], thus increasing feed efficiency. Another benefit of probiotics is that they can increase growt rate and reduce abdominal fat and cholesterol in poultry [4,5]. Probiotics are generally a strain of non-pathogenic bacterials that have a positive effect on the health of the host's and if consumed continuously by poultry, will be able to improve their performance [6].

In the digestive tract of poultry, many beneficial microbes are found, such as from *Lactobacillus* strains. Besides that, there are also many harmful microbes or pathogens, such as *E. coli*. The health status of the digestive tract of poultry will improve if the number of probiotic microbes is sufficient in it [7,8]. Provision of probiotic *Saccharomyces spp.* in the diet can increase the quantity and quality of egg production, calcium retention in the shell, and reduce egg cholesterol levels in ducks [9]. It was reported by [2,3,10], that probiotic supplementation in feed can increase growth performance, nutrient digestibility, and feed efficiency in ducks. The addition of yeast culture (*Saccharomyces cerevisiae*) in the diet can improve egg production performance and reduce egg yolk cholesterol content in laying hens [8,9,11].

Probiotic microbes can suppress the number of pathogenic microbes in the digestive tract of poultry, thereby increasing the number of beneficial microbes. In addition, probiotics also produce natural antibiotics as bacteriocin [12], thus affecting the health and performance of the host [1]. The use of probiotics has been widely applied to poultry, but the responses obtained vary widely [2,3,4,8,9,12,13,14,15,16,17,18,19]. Research on the use of probiotics combined with multi-vitamin-mineral complexes, as a supplement, still needs to be improved in order to obtain efficient and practical poultry production techniques, as well as bio-control of pathogenic bacteria in the digestive tract of poultry.

Minerals and vitamins are essential nutrients for the metabolic and physiological processes of poultry, to support the productive and reproductive performance of poultry [20]. Combining probiotics with a multi-vitamin mineral-mix will have a synergistic effect on ducks. Vitamins can strengthen the immune system in poultry, promote normal metabolism to help fight disease and production stress [21]. Vitamins E and C can act as antioxidants in biological

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systems, which can break the lipid peroxidation chain in cell membranes. Vitamins E and C can be used together synergistically or individually to improve quail health, feed intake, and stimulate quail growth [21].

The study was conducted to examine the ability of probiotic *Saccharomyces spp*. (tape yeast isolate) and multi-vitamin-mineral mix in feed to increase performance and bio-control of *Eschericia coli* bacteria in the intestine of ducks.

Material and Methods

Animal treatments and experimental design. The ducks used in this study were healthy male bali ducks (*Anas sp.*) obtained from a Bali duck breeding business in Badung Regency, Bali Province. Two hundred and forty one-day-old male ducks (*Anas sp.*) were used in a completely randomized design experiment allocated into 4 treatments, namely: P0) rations without Provinal; ration with the addition of 0.1%; 0.2%; and 0.30% Provinal, as treatment P1; P2; and P3, respectively. A total of 240 healthy male Bali ducks 1-d-old (DOD) healthy male ducks (*Anas sp.*) were used in a completely randomized design experiment allocated into 4 treatments. The rations provided were in the mash form consisted of several feed mixtures, such as yellow corn, rice bran, soybeans, fish meal, NaCl, and Provinal. All rations were prepared with the isoprotein content (CP: 16%) and the isoenergy (ME: 2900 kcal/kg). The composition of feed and nutrients in the treatment ration is shown in Table 1. The rations according to the requirements for ducks were based on the recommendations of the [22]. The 240 ducks were divided into four treatment groups, namely: P0) rations without giving a mixture of probiotics and vitamins-minerals (Provinal); Ration with the addition of 0.1% Provinal; 0.2%; and 0.30%, as treatment P1; P2; and P3, respectively. Each treatment was repeated six times each with 10 birds with homogeneous body weight. The size of each cage plot was: 200×1000×50 cm^3 (length×width×height). Each experimental unit was equipped with a feeder and drinking waters, so that birds can eat and drink freely *ad libitum*.

Live performance.

All ducks were placed in one experimental room in cages made of bamboo slats. Feed and drinking water were placed outside the cage, so that the ducks were free to consume and drink. At night, the experimental room was lit by fluorescent lamps. Feed intake (FI), body weight (BW), and live weight gains (LWG) of ducks were calculated per week for eight weeks of observation. Feed consumption was the amount of feed given minus the remaining feed. Calculation of feed consumption based on dry matter (DM) consumption basis. The average weight gain of ducks (ADG) was the difference between the final body weight and the initial weight. Before weighing, the ducks must be fasted with feed for 12 hours, so that the digestive tract was empty of the rest of the feed. During fasting, drinking water was still provided. Feed efficiency (FCR) is the ratio between FI and LWG.

Probiotics and Vitamin-mineral mix (Provinal)

As a source of probiotics, the isolate used was *Saccharomyces spp.*, isolated from Tape yeast [3]. The yeast content of *Saccharomyces spp.* contained in 1 g of culture was $6.71x10^6$ CFU/g. The vitamin-mineral mix used is Vitamin-Mineral B-12. Provinal is a culture mixture of *Saccharomyces spp*. with vitamin-mineral mix with a ratio of 1: 1 (v/v). The ingredients and chemical compositions of the treatment diets are shown in Table 1.

Table 1. The ingredients and calculated nutrient content of the treatment diets

		Treatment Groups			
***Ingredients* (%):**		**P0**	**P1**	**P2**	**P3**
Yellow corn		62.70	62.70	62.70	62.80
Rice bran		22.90	22.80	22.70	22.50
Soybean meal		0.70	0.70	0.70	0.70
Fish meal		13.20	13.20	13.20	13.20
NaCl		0.50	0.50	0.50	0.50
Provinal		0	0.10	0.20	0.30
Total		100	100	100	100
Chemical composition*):					
Metabolizable energy	(kcal/kg)	2903	2901	2900	2900
Crude protein	(%)	16.06	16.05	16.04	16.02
Ether extract	(%)	6.74	6.72	6.71	6.69
Crude fiber	(%)	4.17	4.16	4.15	4.12
Calsium	(%)	1.06	1.11	1.16	1.2
Phosphor	(%)	0.63	0.64	0.65	0.67
Argynine	(%)	1.18	1.18	1.18	1.18
Cystein	(%)	0.33	0.33	0.33	0.33
Leusine	(%)	1.63	1.63	1.63	1.63

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Lysine	(%)	1.18	1.18	1.17	1.17
Methionine+Sistein	(%)	0.75	0.75	0.75	0.75
Tryptophan	(%)	0.17	0.17	0.17	0.17

*) Based on calculations in [23].

Dry matter digestibility (DMD) and organic matter digestibility (OMD).
To calculate the DMD and OMD diets, use the force feeding technique as described by [2]. Ducks were given 100 g of feed (previous observations on ducks, per head per day). The ducks were fasted first from feed for 12 hours, but drinking water was still given, so that the digestive tract of the ducks was empty of leftover feed. The ducks used in this digestibility experiment were placed in individual cages, then the ducks were force-fed with the help of a plastic pipe with a diameter of 1 cm. To accommodate the duck excreta, plastic trays are placed under the cages. The excreta were collected for three days of collection. The excreta were then cleaned of duck feathers and other impurities, then dried in the sun. Furthermore, in the oven at a temperature of 75^0C until constant weight, finely ground and filtered through a 1 mm diameter sieve. Furthermore, samples of feed and feces were brought to the laboratory for analysis of the dry matter (DM) and organic matter (OM) content using the [24] procedure.

Blood sampling and serum analyses. Blood samples were taken at the end of the study (ducks were 8 weeks old), namely two ducks in each experimental unit, so that the total blood samples obtained were 48 samples. Blood samples were taken from the *branchialis* vein at the base of the duck's wing. Blood samples were taken using a 3 ml volume syringe. The total serum cholesterol content was analyzed following the Liebermann-Burchard method [25].

Determination of N-NH_3 concentration, *Escherichia coli* dan *Coliform* in excreta
Excreta samples were taken from the small intestine and large intestine at the end of the study. Determination of N-NH_3 levels using Conway diffusion (26).
Calculation of the population of E. coli and Choliform bacteria in the intestine of ducks. At the end of the experiment, ducks were slaughtered for intestinal sampling. Duck intestines were taken sterile and put in a plastic bag and stored in a cooler. Calculation of the number of *Eschericia coli*, *Choliform*, and total plate count (TPC) bacteria were tested using the Most Probable Number (MPN) method according to the procedure [27].

Statistical analysis. All data were analyzed by means of one-way ANOVA to determine the differences were found, analysis was continued with Duncan's multiple range test at 5% level.

Results

The results were presented in Table 1. Final body weight (FBW), average daily feed intake (ADFI), FCR, DMD, OMD, and total cholesterol concentrations showed significantly different (P<0.05) between treatments. FBW and ADG of ducks that received Provinal treatment (P1, P2, P3) increased significantly different (P<0.05) by supplementeing of Provinal. Likewise, the DMD and OMD of feed increased significantly (P<0.05) in the presence of Provinal supplementation. Provinal supplementation in the diet had no significant effect (P<0.05) on the ADFI. The average FCR value and serum cholesterol concentration in ducks that received Provinal treatment decreased significantly (P<0.05) compared to the control.

Table 1. The ffect of Provinal supplemented in diets on ADG, FCR, feed digestibility and serum cholesterol in ducks.

Variables	Treatments[1]				SEM[2]
	P0	P1	P2	P3	
Initial body weight (g)	53.75a	54.09a	53.94a	54.16a	1.027
Final body weight (g)	1350.25a[3]	1464.08b	1478.36b	1490.37b	23.072
ADG (g/bird/days)	23.15a	25.18b	25.44b	25.65b	0.563
ADFI (g/bird/days)	86.81a	84.10a	83.70a	84.66a	1.049
Feed conversion ratio (ADFI: ADG)	3.75a	3.34b	3.29b	3.30b	0.109
DMD (%)	68.02a	72.19b	73.07b	72.82b	1.208
OMD (%)	70.25a	74.61b	75.49b	75.18b	1.197
Serum cholesterol (mg/dl)	161.09a	144.84b	145.37b	142.52b	3.291

Note:
1. P0): 0% Provinal in diets; P1): 0.10% Provinal in diets; P2): 0.20% Provinal in diets; and P3): 0.30% Provinal in diets, respectively.
2. Standard Error of Treatment Means
3. Means with different superscripts within raw values are significantly different (P<0.05)

An additional of Provinal (P1, P2, P3) in diets resulted significantly different (P<0.05) decreased total cholesterol serum levels in birds (Table 1). The lowest total serum cholesterol content was found in the P3 treatment, which was 142.52 mg/dl or 11.53% lower than control (P0), followed by treatment P1 was 144.84 mg/dl; P2 was 145.37 mg/dl

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(9.76% lower than control). Total cholesterol serum concentrations between treatments P1, P2, and P3, did not show a significantly different (P>0.05).

Table 3 shows that Provinal supplementation in the diet significantly (P<0.05) reduced the total serum cholesterol content in ducks, but had no significant effect (P>0.05) on the number of TPC. More details are presented in Table 3 and Figure 1. The number of *Choliform* bacteria in the intestines of treatment P0 ducks was 5.27 log cfu/g. The number of *Choliform* bacteria in the duck intestines of treatments P1, P2, and P3, were decreased significantly different (P<0.05) rather than control (P0). *E. coli* bacteria in the gut in treatment P0 (control) ducks was 3.98 log cfu/g. Provision of Provinal through diets at the level of 0.1%; 0.2%; and 0.3%, significantly different (P<0.05) decreased *E. coli* bacteria in duck intestines.

Table 3. The effect of Provinal supplementation into the feed on the number of *Coliform* and *Eschericia coli* bacteria in the intestine and ammonia gas levels in the excreta of ducks.

Variables	Treatmens[1]				SEM[2]
	P0	P1	P2	P3	
Microbes in the digestive tract (log CFU/g)					
• *Choliform bacteria*	5.27a	4.39b	4.01b	4.25a	0.013
• *Eschericia coli bacteria*	3.98a	2.74b	2.65b	2.49b	0.392
• Total bacteria count/TPC	9.39a	8.75a	8.49a	8.61a	0.695
N-NH_3 (m.Mol)	41.95a	32.51b	33.06b	31.94	3.072

Note:
1. P0): 0% Provinal in diets; P1): 0.10% Provinal in diets; P2): 0.20% Provinal in diets; and P3): 0.30% Provinal in diets, respectively.
2. Standard Error of Treatment Means
3. Means with different superscripts within raw values are significantly different (P<0.05)

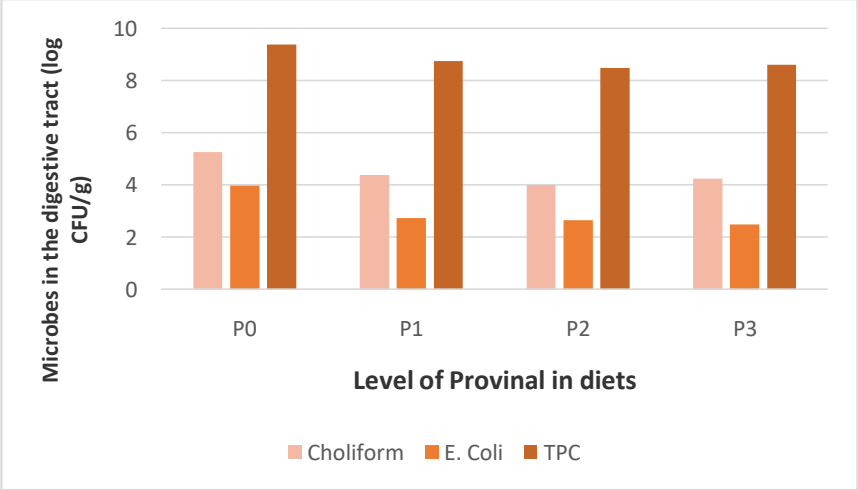

Figure 1. Effect of Provinal supplementation in the diet on the *Choliform* and *E. coli* bacteria in the intestine of ducks.

The concentration of ammonia gas (N-NH_3) excreta in ducks decreased significantly (P<0.05) with increasing administration of Provinal in the feed (Table 3). The concentration of N-NH_3 in the duck excreta in Group P0 was 41.95 m.mol. Provision of Provinal to ducks in treatments P1, P2, and P3 were decreased significantly different (P<0.05), namely: 22.50%; 21.19%; and 23.85% compared to control (treatment P0). More detail was presented in Figure 2.

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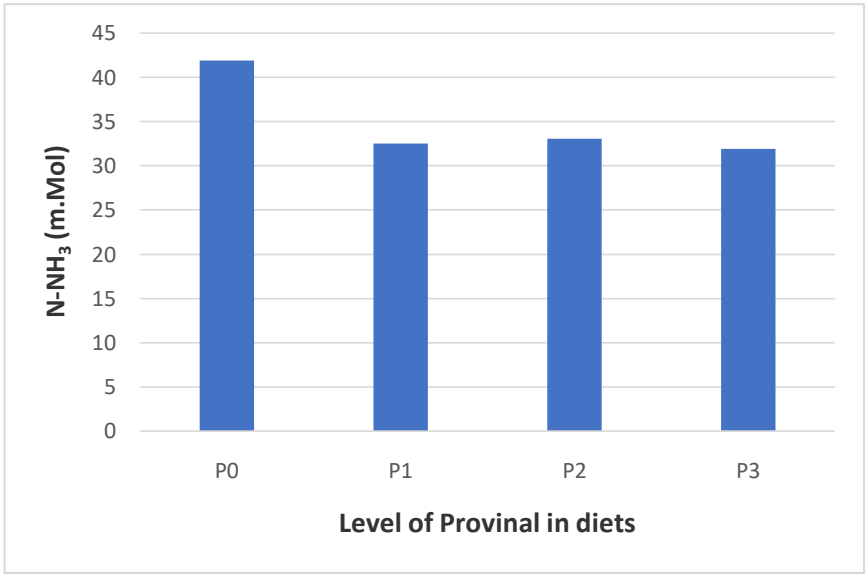

Figure 2. The effect of giving Provinal in the ration to the concentration of N-NH_3 in duck excreta.

Discussion

Provinal supplementation in feed did not affect ADFI. This is logical, because the ME content in the ration is the same (Table 1). Ducks will consume feed to meet energy needs. As reported by [28], that supplemented of probiotics in diets has no effect on FI. Provinal supplementation in the diet will have a positive synergy. Multi-vitamin-mineral will increase the host's immune system, as well as probiotics. The mixture of these two ingredients in the diet will be able to increase enzymatic activity, so that it has an impact on increasing feed digestibility. The results of this study prove that Provinal supplementation in feed can significantly increase final body weight, ADG, feed efficiency, DMD, and OMD. Minerals were needed by microbes for the synthesis of body biomass and also to support the growth of chickens. As reported by [29], increased levels of Ca in the feed showed an increasing trend of chicken productivity. Reported by [3], that supplementation of probiotic *Saccharomyces spp*. into the diet of ducks can increase ADG and feed digestibility, so that feed efficiency increases. It was reported by [26] that supplementation of *Saccharomyces spp* isolated from tape yeast in laying hens rations, could increase DMD, OMD, and protein. The same thing was reported by [2] that Supplementation of yeast *Saccharomyces spp*. isolated from buffalo rumen can increase ADG and DMD in ducks. Increased nutrient digestibility will affect feed efficiency. Administration of *L. acidophilus* and *B. subtilis* as probiotics in feed alone or in combination can increase beneficial bacteria in the intestines, DMD, and FCR, thus having an impact on increasing chicken performance [10,30]. As reported by [10], probiotic-mix supplementation in feed significantly increased feed digestibility in pigs. Contrary to research, [30] reported that the administration of probiotic *B. subtilis* did not provide a significant response in rabbits.

The inclusion of Provinal in diet (treatments P1, P2, and P3) significantly reduced total serum cholesterol. It was reported by [31] that the supplementation of probiotic *Lactobacillus* in the diet can reduce total serum cholesterol in broiler. Probiotics are effectively able to reduce egg yolk cholesterol levels [8]. Contrary to the research of [32,33] that the use of probiotics has no effect on blood lipid profiles. The differences in the inconsistent results were caused by differences in the number of probiotic microbes used, and the age of the poultry [12]. According to [34], it is possible that differences in inconsistent results from several researchers depended on several factors, such as the composition of the microbial *strain* (single *strain* or multi-*strain*), concentration of supplementation, method of application, frequency of application, feed quality, age of poultry, cleanliness of the cage environment, and environmental stress.

Probiotics can maintain the balance of microbes in the digestive tract of poultry, namely through a competitive exclusion mechanism, namely competition between pathogenic bacteria and probiotic microorganisms, so that pathogenic bacteria cannot live in the digestive tract and will come out with excreta [1]. Probiotics can maintain the balance of the microbial population in the digestive tract of poultry [35]. According to [36], the balance of microbes in the digestive tract of poultry occurs when the composition consists of 85% beneficial microbes and 15% pathogenic microbes. Probiotic microbes can create a balance of microflora in the digestive tract of poultry, because the presence of lactic acid bacteria in the digestive tract of poultry can create an acidic atmosphere, thereby suppressing the population of pathogenic bacteria [37]. The results of the study [38,39] reported that supplementation of fermented feed products in the diet can suppress the population of pathogenic bacteria (*Salmonella* and *Campylobacter*) in the digestive tract of broilers.

The same study reported by [40] that supplementation of probiotik in the diet can reduce the population of pathogenic bacteria in the digestive tract of rabbits. Yeast *S. cerevisiae* can stimulate the growth of beneficial aerobic and anaerobic groups of bacteria in the rabbit intestine. In broiler chickens, administration of a mixture of *Saccharomyces cerevisiae* with *Streptoccus faecum* can reduce the population of *Eschericia coli* up to 50% [41]. Similary to the study [30], that supplementation of *Lactobacillus* probiotics in the diet can suppress the population of *Choliform* and *Eschericia choli* bacteria in the rabbit intestine compared to the control diet (without probiotics). Supplementation of probiotic-mix in feed can increase population of *Lactobacillus* bacteria and suppress the population of *E. coli* in pig excreta [10], reducing the number of *Choliform* and *E.choli* bacteria in chicken intestines [26].

Provinal supplementation in the diet (P1, P2, P3) can reduce ammonia gas concentrations in the excreta. The decrease in the concentration of ammonia gas in excreta was caused by probiotics that can suppress urease activity, so that uric acid decreases, and some uric acid is used for microbial protein synthesis [42]. Reported by [10], that 0.30% probiotic supplementation in pig, duck, and chicken feed, can significantly increase nutrient digestibility, suppress pathogenic bacteria, and ammonia gas emissions [10,5,12,19,43]. The same thing was reported by [26] that supplementation of the probiotic *Saccharomyces spp*.N-2 in feed in laying hens can reduce the ammonia gas content in chicken excreta. Reported by [10] that probiotic-mix supplementation in feed significantly reduced the ammonia gas content in pigs.

Conclusion

It can be concluded that the inclusion of 0.10-0.30% Provinal in the diet can stimulate duck growth, feed efficiency, and nutrient digestibility. On the other hand, it significantly reduced the concentration of cholesterol in serum, N-NH_3 in excreta, total population of *Choliform* bacteria and *E. coli* in ducks.

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