# Effect of Bacillus amyloliquefaciens

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Research Article

# Effect of *Bacillus amyloliquefaciens* as a Probiotic on Growth Performance Parameters of Pitalah Ducks

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## Abstract

**Background and Objective:** Live probiotic feed additives can balance microorganism populations in the digestive tract. This study aimed to investigate the effects of the probiotic *Bacillus amyloliquefaciens* on growth performance and feed efficiency of Pitalah starter ducks. **Methodology:** A completely randomized design was used in this study. Three different doses of *B. amyloliquefaciens* (1000, 2000 and 3000 ppm) were dispensed in the drinking water of 1 day old Pitalah ducks (N = 25 duck per dose) and each treatment was replicated five times, control birds were given 0 ppm (N = 25 duck). Feed consumption and conversion, body weight gain, income over feed cost, total colony count of *Bacillus* sp., in the small intestines and acidity in small intestine were measured. **Results:** The results showed that all three doses significantly decreased feed consumption, feed conversion and small intestinal acidity ($p<0.05$) and increased income over feed cost and total small intestinal *Bacillus* sp., colony counts. There was non significant change in weight gain ($p>0.05$). **Conclusion:** It is concluded that addition of 2000-3000 ppm of *B. amyloliquefaciens* to the drinking water of Pitalah ducks can improve the feed efficiency by >15% and provide economic benefits for farmers raising starter ducks.

**Key words:** *Bacillus amyloliquefaciens*, probiotics, feed efficiency, performance, Pitalah ducks

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Raising ducks for eggs and meat provides relatively affordable and available sources of animal protein. Previous study indicated that raising poultry provides 20-30% of the total animal protein consumed in developing countries[1]. Ducks have an advantage over other poultry in that they are omnivorous, not only eating seeds, grass and other vegetation but also small animals, like fish and snails and can thrive on the high-fiber, low-quality food better than chickens. Duck raising produces 27.900 t or 0.18% of all poultry meat in Indonesia, with the majority being supplied by broiler chickens[2].

The Pitalah duck is a unique type of duck native to West Sumatra, Indonesia, with a dwindling population and genetic purity[3]. The population of ducks farmed in Indonesia dropped 2.14% from 2010-2011 mainly because raising methods have not yet been modernized and due to the scarcity of feed. The availability of cheap, quality feed has dwindled in recent years because much of this food is being increasingly used for human consumption[4]. In poultry production, feed prices generally comprise 60-80% of the cost of production[5]. Feed price is also major factor determining the success of the poultry business, particularly for ducks that require more feed than chickens. Because of fluctuations in the availability and price of feed, intensive or semi-intensive duck raising businesses tend to fail. Prasetyo *et al.*[6] reported that the average feed conversion of 6 weeks old ducks is 4.13-4.31. Hence, the high cost of feed makes it almost impossible for farmers to farm ducks intensively.

The growth of animals during the early (starter) period is very dependent on feed rations. To improve the efficiency of feed utilization in cattle; many breeders add probiotics to feed during the initial growth period. However, there are far fewer studies on the administration of probiotics to ducks than to the broiler. Probiotics are live microorganisms which confer health benefits to the host when administered in adequate amounts[7,8]. Probiotics can improve egg weight, feed efficiency, yolk color, egg shell quality, pathogen inhibition[9,10] and have the ability to lower serum cholesterol levels[11].

*Bacillus* sp., are aerobic bacteria capable of forming endospores. Recently, these microorganisms were shown to be promising probiotics as they were able to survive the digestive process, develop in the digestive tract and be excreted through fecal matter[12-14]. In particular, *B. amyloliquefaciens* potently produce several extracellular enzymes, such as α-amylase, cellulase, metalloproteases and other proteases, which improve digestibility and absorption of nutrients, as well as overall intestinal immune function[15,16]. Additionally, *B. amyloliquefaciens* produce bacteriocins subtilin and barnase[17,18]. *Bacillus amyloliquefaciens* supplementation has been shown to exert protective effects on the growth performance and immune function of broiler chickens under immunological stress induced by lipopolysaccharide challenge from *Escherichia coli*[19]. Moreover, this strain has been shown to survive in the small intestines of laying broiler chickens for 32 days at a colony count of $18\times10^{-7}$ CFU $mL^{-1}$ reduce feed consumption by 0.9% and increase egg weight by 5.39%[20]. Therefore, the present study investigated the potential benefits of using *B. amyloliquefaciens* as a probiotic supplement in Pitalah duck raising.

## MATERIALS AND METHODS

**Sample collection:** Local ducks Pitalah were used in this study. One day old as many as 100 ducks were obtained from Payakumbuh, West Sumatra, Indonesia, with the male sex were purchased in duck farming folk. Ducks were housed in 80×60×60 cm box cages, with 5 ducks per box. Each box was supplied with a 60 W incandescent lamp for light and warmth. Feed and drinking water were supplied *ad libitum* for the entire 6 weeks study period. Rations were comprised of corn flour, bran, fish flour, soybean meal, coconut oil, bone meal and top mix. The nutrient (%) and metabolic energy (kcal $kg^{-1}$) content of the bird feed is shown in Table 1. Fresh drinking water was supplied every day and *B. amyloliquefaciens* was administered in the drinking water, starting when birds were 1 day old.

**Total colony count of *Bacillus* sp., from small intestines:** The total of *Bacillus* sp., at the end of the 6 weeks treatment period was determined by removing the small intestines of one Pitalah duck per box and calculating the CFU using a method of dilutions and total plate counts as described by Cappuccino and Sherman[21] and Hadioetomo[22]. Briefly, the contents (~1 g) of the small intestine of each Pitalah duck were serially diluted in 1:10 increments from $10^{-1}$ to $10^{-12}$ in distilled water (12 total dilutions). Approximately $10^{-12}$ dilution of intestinal contents from each (1 mL) was inoculated onto petri dishes filled with *Bacillus* sp., selective medium and incubated at room temperature for 24 h.

**Experimental design:** A completely randomized study design was used to examine the effects of supplementing the drinking water of Pitalah ducks with three different doses of *B. amyloliquefaciens* (1000, 2000 and 3000 ppm); (N = 25

Table 1: Composition, nutrient content and metabolic energy of Pitalah duck feed

| | *Bacillus amyloliquefaciens* dose (ppm) | | | |
|---|---|---|---|---|
| Feed materials | 0 | 1000 | 2000 | 3000 |
| Corn (%) | 52.5 | 52.5 | 52.5 | 52.5 |
| Bran (%) | 14 | 14 | 14 | 14 |
| Fish flour (%) | 15 | 15 | 15 | 15 |
| Soybean meal (%) | 16 | 16 | 16 | 16 |
| Coconut oil (%) | 0.5 | 0.5 | 0.5 | 0.5 |
| Bone meal (%) | 1.5 | 1.5 | 1.5 | 1.5 |
| Top mix (%) | 0.5 | 0.5 | 0.5 | 0.5 |
| Total (%) | 100 | 100 | 100 | 100 |
| Crude protein (%) | 20.41 | 20.41 | 20.41 | 20.41 |
| Crude fat (%) | 5.24 | 5.24 | 5.24 | 5.24 |
| Crude fiber (%) | 4.57 | 4.57 | 4.57 | 4.57 |
| Calcium (%) | 1.10 | 1.10 | 1.10 | 1.10 |
| Phosphorus (%) | 0.78 | 0.78 | 0.78 | 0.78 |
| Energy metabolism (kcal $kg^{-1}$) | 2904.05 | 2904.05 | 2904.05 | 2904.05 |

duck per dose) or 6 weeks; control birds were given 0 ppm (N = 25 ducks). Growth performance variables; feed consumption and conversion, body weight gain, Income Over Feed Cost (IOFC) and small intestinal total *Bacillus* sp., CFU and acidity were measured. Growth performance data were collected once a week by weighing ducks and any remaining food once a week. Each treatment was replicated five times.

**Statistical analysis:** All data were analyzed by use analysis of variance (ANOVA), using a general linear model procedure on SPSS software version 16.0. Duncan's multiple range test was used for determination of differences between treatment dose means[23].

## RESULTS AND DISCUSSION

**Effect of *B. amyloliquefaciens* on growth performance parameters:** The effects of *B. amyloliquefaciens* on feed consumption and conversion, body weight gain, IOFC and small intestinal total *Bacillus* sp. CFU and acidity of Pitalah ducks are shown in Table 2 and 3. At the end of the 6 weeks study period, it is found that supplementation with all three doses of *B. amyloliquefaciens* significantly decreased feed consumption, feed conversion and small intestinal acidity ($p<0.05$) and significantly increased the IOFC and total CFU of *Bacillus* sp., in the small intestines ($p<0.05$) compared to control ducks; however, weight gain was not significantly different ($p>0.05$).

**Feed consumption:** Analysis of variance indicated that all three doses of *B. amyloliquefaciens* significantly decreased feed consumption compared to controls ($p<0.05$). Table 2 shows that the feed consumption of 6 weeks old starter ducks was significantly reduced as the dose of probiotic increases and apparently plateaus somewhere between 2000 and 3000 ppm. These results indicated that supplementation with *B. amyloliquefaciens* leads to more efficient feed consumption of Pitalah ducks.

These results were likely caused, at least in part, by the beneficial production of multiple enzymes by *B. amyloliquefaciens*, including α-amylase, α-acetolactate decarboxylase, β-glucanase, maltogenic amylase, urease, protease, xylanase, chitinase, phytase, cellulase, hemicellulase and lipase[24-27]. *Bacillus amyloliquefaciens* strain 10A1 has also been found to produce the enzyme β-mannanase[28]. Production of these enzymes in the small intestine of ducks would increase their ability to digest and absorb a larger percentage and variety of nutrients in their feed. Hence, ducks given this bacterium require less feed compared to ducks that were not given the probiotic. Furthermore, increasing the amount of *B. amyloliquefaciens* also likely improves the quality of the intestinal microflora, increasing the population of helpful bacteria and depressing the population of harmful species. This would also improve the digestive processes and efficiency of feed absorption[29].

Sjofjan[30] showed that the intestinal surface area of chickens given *Bacillus* sp., is greater than that of those not given this probiotic. Mountzouris *et al.*[10] showed that probiotics can change the movement of mucin, microbial populations and the cecal microflora composition in chicken small intestines, improving their health, function and absorption of nutrients from feed. However, probiotics must be given in sufficient quantities to have a beneficial effect. Nirmalasanti[31] stated that probiotics do not work well if the dose is too small, the targeted environment is not sufficiently clean or the initial microflora composition is not appropriate

Table 2: Average feed consumption, conversion, body weight gain and IOFC of Pitalah ducks treated with *Bacillus amyloliquefaciens* for 6 weeks

| *Bacillus amyloliquefaciens* dose (ppm) | Feed consumption (g/bird) | Body weight gain (g/bird) | Feed conversion | IOFC ($/bird) |
|---|---|---|---|---|
| 0 | 3994.34[a] | 917.60 | 4.37[a] | 0.63 |
| 1000 | 3743.05[b] | 920.01 | 4.08[b] | 0.74 |
| 2000 | 3390.12[c] | 922.60 | 3.68[c] | 0.91 |
| 3000 | 3330.28[c] | 923.40 | 3.61[c] | 0.92 |

Data presented as the mean of 5 biological replicates, [a-c]Values in the same column with different superscript letters are significantly different ($p<0.05$)

Table 3: Average total *Bacillus* sp., colony count and acidity (pH) in the small intestines of Pitalah ducks treated with *Bacillus amyloliquefaciens* for 6 weeks

| *Bacillus amyloliquefaciens* dose (ppm) | Total *Bacillus* sp., colony count (log CFU $mL^{-1}$) | Acidity (pH) |
|---|---|---|
| 0 | 13.91[a] | 6.14[a] |
| 1000 | 14.37[b] | 5.68[b] |
| 2000 | 14.84[c] | 5.55[c] |
| 3000 | 14.85[c] | 5.53[c] |

Data presented as the mean of 5 biological replicates, [a-c]Values in the same column with different superscript letters are significantly different ($p<0.05$)

for the animal or has degraded due to poor storage. Interestingly, even the lowest dose of *B. amyloliquefaciens* in the current study significantly reduced the amount of feed required by ducks and continued to significantly increase feed efficiency up to 2000 ppm. On average, Pitalah ducks given 2000 ppm of *B. amyloliquefaciens* ate 3390.12 g of feed per bird after 6 weeks, which is only slightly higher than that reported by Rositawati *et al.*[32] (3094.46 g $bird^{-1}$) in ducklings fed 0.6% Temulawak (*Curcuma xanthorrhiza*) over a similar time period.

**Body weight gain:** Analysis of variance revealed that dosing drinking water with *B. amyloliquefaciens* did not have a significant effect on weight gain, nor there was a significant trend as the concentration of probiotic increased (Table 2). This indicates that all ducks in the current study were able to acquire the necessary nutrition from their feed to reach the appropriate developmental size. Weight gain is an indication of the efficiency with which an animal converts its feed. If large amounts of feed are consumed with little weight gain, digestion was incomplete. Weight gain is also influenced by the health of the ducks, gender, environment temperature and food quality. These factors being equal, an increase in weight closely correlates with feed consumption. Current results indicated that supplementation with *B. amyloliquefaciens* reduced feed consumption without a detrimental effect on weight gain due to the probiotic's ability to aid digestion and absorption of feed, making more nutrients are available for tissue growth, energy and weight gain instead of being excreted in the feces. The average weight gain over the 6 weeks study period was 922.60 g $bird^{-1}$, which was more than that observed in starter ducks fed 0.6% Temulawak (*Curcuma xanthorrhiza*) after 6 weeks (732.7 gained per bird)[32].

**Feed conversion:** Analysis of variance showed that administration of *B. amyloliquefaciens* significantly reduced the feed conversion as the dose of probiotic increased ($p<0.05$) and plateaus between 2000 and 3000 ppm in 6 weeks old Pitalah ducks (Table 2). This is due to the fact that feed consumption decreased as the probiotic dose increased with no corresponding reduction in weight gain. Feed conversion is the ratio of feed consumed and weight gained over a particular time and the quality of the feed significantly influences this conversion rate[33]. High-quality, palatable feed with an appropriate balance of vitamins and nutrients results in a low feed conversion ratio, while unpalatable low-quality feed results in a high feed conversion[34]. The level of feed conversion is highly dependent on the balance between the metabolic energy and nutritional value of the feed, particularly the protein and amino acid content[35]. *Bacillus amyloliquefaciens* can reduce feed conversion with its production of multiple enzymes that aid digestion, such as proteases and cellulases, thereby increasing the feed efficiency. Furthermore, *B. amyloliquefaciens* has been shown to be a beneficial feed additive for broiler chickens, where 20 g $kg^{-1}$ showed the highest efficiency[36]. The average feed conversion for starter ducks given 2000 ppm *B. amyloliquefaciens* for 6 weeks was 3.68, which is similar to that obtained by Riswandi *et al.*[37] (3.56), who gave local starter ducks a combination of Starbio and EM-4 in their feed and drinking water.

**IOFC:** The IOFC measures the difference between the income from the business and the cost of the feed[38] and is a product of animal production volume and market price. Production volume is in turn influenced by feed price, feed consumption, final body weight, digestion and absorption of feed (i.e., feed conversion), morbidity and mortality[39]. Though the IOFC for 6 weeks old Pitalah ducks increases with the addition of 0 ($ 0.63/bird) to 3000 pmm ($ 0.92/bird) *B. amyloliquefaciens* (Table 2), this trend was not significant for any of the doses

tested. The highest IOFC was for 3000 ppm, indicating this probiotic dose was best in the current study because less feed was consumed by this group. Lower feed consumption means lower feed costs and a higher IOFC per duck. However, since the difference in IOFC between 2000 and 3000 ppm is so small, both doses can be considered equally good in practical terms.

**Total *Bacillus* sp. colony count in the small intestines:** Table 3 shows the range of *Bacillus* sp., population numbers in the small intestines of 6 weeks old Pitalah ducks significantly increase in a concentration-dependent manner and plateaus between 2000 and 3000 ppm doses of *B. amyloliquefaciens*. *Bacillus* sp., are rod-shaped, lactic acid and endospore-producing, Gram-positive bacteria that can survive between pH 4-7.5 and at 30-45°C[40]. These species produce a 27.85 mm clear zone in CMC medium and show Cx and C1 cellulase activity in high-fiber media (23.57%) of 0.488 and 1.200 U $mL^{-1}$. Importantly, spores of *Bacillus* sp., can survive pasteurization[24]. Farmer and Lefkowitz[40] reported that *Bacillus* sp., can thrive in the digestive tract of ducks as the conditions provide sufficient nutrients and an ideal pH, temperature and level of moisture. Small intestinal pH[41] can range from 5.59-6.62, which is appropriate for acidophilic *Bacilli*. Additionally, these probiotics could stabilize the intestinal ecosystem of animals by enhancing the growth of beneficial bacteria and competing with pathogenic bacteria in the intestine[42,43]. Supplementation of *B. amyloliquefaciens* as a Direct Fed Microbials (DFM) has been reported to increase the concentration of *Lactobacillus*[9,44] and reduce the concentration of *E. coli* in the cecal digesta of broilers.

**Small intestine acidity:** Analysis of variance indicated that adding *B. amyloliquefaciens* to the drinking water of Pitalah ducks for 6 weeks significantly decreased the pH of the small intestines ($p<0.05$). The pH range of the small intestines significantly decreased as the dose of probiotic increased and remained unchanged from 2000-3000 ppm (Table 3). These results are most likely because the increase in *Bacillus* sp., population numbers will lead to an increase in the production of organic acids by these acidophilic bacteria, followed by a consequent decrease in pH in the digestive tract[45]. Additionally, lactic acid and bacteriocin produced by *B. amyloliquefaciens* are known to cause a severe drop in intestinal pH which is favorable to *Lactobacilli* colonization and suppression of *E. coli* growth[46].

## CONCLUSION

It is concluded that administration of *B. amyloliquefaciens* to the drinking water of starter Pitalah ducks for 6 weeks decreased feed consumption and improved the efficiency of the ration, with a >15% decrease in feed conversion, due to an increase in small intestinal CFU and acidity. This, in turn, resulted in an increase in IOFC but has no influence on weight gain.

## SIGNIFICANCE STATEMENTS

The current study investigated the effect of *Bacillus amyloliquefaciens* supplementation on Pitalah ducks in West Sumatra, Indonesia, for the first time. The results reported here revealed that dietary administration of *B. amyloliquefaciens* as a probiotic can significantly improve feed efficiency, growth performance parameters and investment over feed costs of starter Pitalah ducks.

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