



## Effect of Biofeed-H-lysine Supplementation in Feed on Performance, Blood Lipid Profile and Intestinal Pathogenic Bacteria in Ducklings

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### ABSTRACT

The study aimed to determine the effect of "Biofeed-H-lysine" on production performance, blood lipid profiles and pathogenic bacteria in the intestines of ducks. A total of 240 male Bali ducklings (*Anas sp.*) were randomly divided into four experimental groups with 6 replicates and each replicate with 60 ducklings. Ducklings in Group 1 (n=60) served as healthy controls and were fed a basic diet without the addition of Biofeed-H-lysine. Ducklings in Groups 2, 3 and 4 were fed with addition of 1, 2 and 3% Biofeed-H-lysine per kg of feed. The results showed that the body weight gain, and feed efficiency in the group of ducklings that received biofeed-h-lysine were significantly different ( $P<0.05$ ) higher than the control. Supplementation of 2-3% Biofeed-H-lysine in feed significantly ( $P<0.05$ ) reduced blood serum cholesterol and triglyceride levels, and significantly ( $P<0.05$ ) reduced the population of *Coliform* and *E. coli* bacteria in the intestines of ducks. It can be concluded that supplementation of 2-3% biofeed-H-lysine in feed can improve performance, as well as reduce serum cholesterol and pathogenic bacteria in the intestines of ducks.

**Key words:** Ducklings, Lysine, Pathogenic bacteria, Probiotics

### INTRODUCTION

The implementation of biotechnology products as feed supplements for duck feed which is able to increase the quantity and quality of products with a high level of feed efficiency is a future policy strategy that is highly expected as a substitute for the use of antibiotics which have been banned since 2018. In the tofu processing industry, around 35-40% solid waste is produced (Faisal et al. 2016) or around 1,024 million tonnes/year (Ajijah et al. 2019). The limiting factor for using tofu waste as poultry feed is the high crude fiber content, ranging from 19-24.03% which can reduce poultry productivity (Mulia et al. 2015; Sari et al. 2016; Nurhayati et al. 2019).

Biofeed-H-lysine is a feed biotechnology product in powder form based on tofu waste and carrots which is fermented with probiotic inoculants which are high in protein (22.96±1.37%), lysine (2.15±0.014%) and contains probiotic microbes ( $10^7$  cfu/g) (Bidura et al. 2023). The combination of phyto-genic additives with probiotics in feed significantly increases nutrient intake, feed efficiency, and jejunal villi height in laying hens (Hedayati and Manafi 2018; Hidayat et al. 2021).

Probiotics are beneficial microorganisms that enhance the immune system, produce organic acids so that the intestinal microflora is always in a balanced state, thus improving the absorption of nutrients in the host's digestive tract. Good absorption of nutrients in the digestive tract can affect productivity in poultry (Priastoto et al. 2016; Kalita et al. 2023). Microorganisms that are often used as probiotics are strains of *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Pediococcus* and *Yeast* (Soccol et al. 2010). The use of probiotics from the yeast *Saccharomyces spp.* in the diet can lead to increased production performance, increased feed digestibility, feed efficiency, and egg production (Zurmiati et al. 2014; Jannah et al. 2022). Supplementation of probiotics *Lactobacillus parabuchneri*, *Lactobacillus buchneri*, *Lactobacillus harbinensis*, and *Lentilactobacillus parabuchneri* at a level of 1.0mL/L via drinking water, significantly increases feed efficiency and carcass quality in broilers (Susalam et al. 2024).

Based on this, researchers are interested in studying the effect of fermented feed products (Biofeed-H-lysine) as feed supplements to promote growth and bio-control of pathogenic bacteria in ducklings.

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## MATERIALS AND METHODS

### Ethical approval

This experiment was conducted using 240 two-week-old male Bali ducks (*Anas sp.*) with the approval of the Animal Ethics Commission, Faculty of Veterinary Medicine, Udayana University, and Denpasar, Indonesia.

### Experimental design

Ducks were provided with standard duck feed (Table 1) and given *ad libitum*. Ducklings (n=240) were randomly divided into four experimental groups with six replicates and each replicate with sixty birds. Ducklings in Group 1 (n=60) served as healthy controls and were fed a basal diet without the addition of Biofeed-H-lysine. Ducklings in Groups 2, 3 and 4 were fed with the addition of 1, 2, and 3% Biofeed-H-lysine per kg of feed. The feed given was formulated based on nutritional requirements for ducklings for a 10-week trial. The composition of feed and nutrients is presented in Table 1.

**Table 1:** The composition of feed and nutrients in ducklings aged 2-10 weeks

Basal Diets		Compositions
Ingredients (%):		
		54.10
		13.10
		13.10
		8.00
		10.32
		0.50
	Total	100
Chemical composition:		
Metabolizable energy	(kcal/kg)	2901
Crude protein	(%)	18.0
Crude Fiber	(%)	5.12
Ether Extract	(%)	7.30
Calcium	(%)	2.93
Phosphorous	(%)	1.10
Arginine	(%)	0.63
Lysine	(%)	1.16
Metionin+sistein	(%)	0.68
Tryptophan	(%)	0.20

The variables observed were: final body weight (FBW), body weight gain (LWG), feed consumption (FC), and feed efficiency (comparison between FC and LWG). Determination of *Escherichia coli* and *Coliform* populations followed the procedure of Sudatri (2021) by scatter method in EMBA media, namely 5g of digesta sample was put into an Erlenmeyer containing 0.1% peptone water solution with a volume of 45ml, resulting in a  $10^{-1}$  dilution. Planting at a dilution level of  $10^{-1}$  to  $10^{-7}$  was to count bacterial colonies that grow using the cup count method (30-300 colonies). The cholesterol content in the serum was evaluated from the blood sample. Cholesterol levels were analyzed according to the Lieberman-Burchard method (Lieberman and Burchard 1980).

### Statistical analysis

The data obtained were analyzed with one-way analysis of variance, if there was a significant difference ( $P < 0.05$ ) between the treatments, then it was continued with Duncan's multiple range test.

## RESULTS AND DISCUSSION

Table 2 shows the growth and feed efficiency of ducklings fed biofeed-h-lysine from 2-10 weeks of age. The addition of Biofeed-H-lysine in feed significantly ( $P < 0.05$ ) improved duckling performance. The addition of 2-3% Biofeed-H-lysine in the feed significantly ( $P < 0.05$ ) increased the FBW of ducks, namely 7.83 and 8.79% higher than the control. The duck group that received feed containing 2 and 3% Biofeed-H-lysine, their LWG for 8 weeks of observation, were 10.45 and 11.64% significantly ( $P < 0.05$ ) higher than the control (without Biofeed-H-lysine).

The addition of Biofeed-H-lysine at a level of 2 and 3% in feed significantly ( $P < 0.05$ ) increased feed efficiency, respectively 9.28 and 11.14% higher than the control. The addition of 1% Biofeed-H-lysine to feed had no significant effect ( $P > 0.05$ ) on feed efficiency.

The impact of adding Biofeed-H-lysine to duckling feed from 2-10 weeks of age on *Coliform* and *E. coli* populations in the jejunum is presented in Table 3. Addition of 1% Biofeed-H-lysine to feed did not have a significant effect ( $P > 0.05$ ) on the population of *Coliform* and *E. coli* in the duckling jejunum. However, the addition of 2 and 3% Biofeed-H-lysine in the feed significantly ( $P < 0.05$ ) reduced the population of *E. coli* bacteria, namely 33.48 and 35.53% significantly ( $P < 0.05$ ) lower than the control. The same thing happened to the number of *Coliforms* in the jejunum of ducks which decreased significantly ( $P < 0.05$ ), namely: 13.99 and 17.01% lower compared to the control diet.

Table 4 presents the blood lipid profile (total cholesterol, LDL, HDL, triglycerides) of ducklings given additional Biofeed-H-lysine supplements in feed from 2-10 weeks of age. The addition of biofeed-h-lysine had a significant ( $P < 0.05$ ) effect on the duckling blood lipid profile. The total blood serum cholesterol levels of the ducks that were given 2 and 3% Biofeed-H-lysine in the feed decreased significantly ( $P < 0.05$ ), namely: 12.32 and 18.13% lower than the control. Likewise, blood serum triglyceride levels experienced a significant decrease ( $P < 0.05$ ), namely: 15.82 and 15.55% lower than the controls.

The addition of 2-3% Biofeed-H-lysine in feed significantly increases LWG and feed efficiency. This is due to the high content of the amino acid lysine in Biofeed-H-lysine. According to Jiang et al. (2021), the content of the amino acid lysine in the ration is very important, because the amino acid lysine is the first limiting amino acid in poultry and is very suitable for improving the quality of low-protein feed. As reported by Liao et al. (2015) and Batool et al. (2021), the amino acid lysine is involved in physiological processes, particularly in body protein synthesis, and helps digestion and utilization of nutrients (Zeng et al., 2013). Besides that, Biofeed-H-lysine is a fermented feed product, where the protein and mineral content of fermented products is higher than non-fermented (Mulia et al. 2015; Nurhayati et al. 2019).

*Saccharomyces cerevisiae* can break down complex carbohydrates into simple compounds, increasing enzymatic activity, digestibility, and absorption of nutrients (Saferi et al. 2005; Zurmiati et al. 2014).

**Table 2:** Performance of male Bali ducklings aged 2-10 weeks fed with Biofeed-H-lysine supplementation

Variables	Level of addition of Biofeed-H-lysine in feed (%)				SE
	0	1	2	3	
Initial body weight (g/head)	351.15	349.94	350.73	351.53	2.795
FBW (g/head)	1418.32 <sup>a</sup>	1435.74 <sup>a</sup>	1529.37 <sup>b</sup>	1542.93 <sup>b</sup>	21.062
LWGs (g/head/56 days)	1067.17 <sup>a</sup>	1085.80 <sup>a</sup>	1178.64 <sup>b</sup>	1191.40 <sup>c</sup>	19.318
FC (g/head/56 days)	4599.50	4658.08	4608.48	4563.06	48.752
FC (g/head/days)	82.13	83.18	82.29	81.48	0.815
FCR	4.31 <sup>a</sup>	4.29 <sup>a</sup>	3.91 <sup>b</sup>	3.83 <sup>b</sup>	0.083

Means with a common superscript within a row are significantly not different at probability  $P < 0.05$ ; FCR=Feed conversion ratio (FC/LWG)

**Table 3:** Impact of Biofeed-H-lysine in duckling feed from 2-10 weeks of age on *Coliform* and *E. coli* populations in the intestine

Variables	Level of addition of Biofeed-H-lysine in feed (%)				Normal population
	0	1	2	3	
Bakteri <i>E. coli</i> (cfu/g)	$9.26 \times 10^3 \pm 0.25 \times 10^{3a}$	$8.72 \times 10^3 \pm 0.17 \times 10^{3a}$	$6.16 \times 10^{3b} \pm 0.12 \times 10^{3b}$	$5.97 \times 10^3 \pm 0.15 \times 10^{3b}$	$10^4 - 10^5$
<i>Coliform</i> bacteria (cfu/g)	$7.29 \times 10^4 \pm 0.16 \times 10^{4a}$	$7.13 \times 10^4 \pm 0.13 \times 10^{4a}$	$6.27 \times 10^4 \pm 0.11 \times 10^{4b}$	$6.05 \times 10^4 \pm 0.14 \times 10^{4b}$	$4.0 \times 10^6$ to $9.4 \times 10^6$

Means with a common superscript within a row are significantly not different at probability  $P < 0.05$ ; cfu=Colony forming unit

**Table 4:** Blood lipid (mg/dL) profile (total cholesterol, LDL, HDL, triglycerides) of male Bali ducklings fed with the addition of Biofeed-H-lysine supplements

Variables	Level Biofeed-H-lysine dalam pakan (%)				SEM
	0	1	2	3	
Total cholesterol	198.35 <sup>a</sup>	190.27 <sup>a</sup>	173.92 <sup>b</sup>	162.38 <sup>b</sup>	3.052
High density lipoprotein	65.91	67.38	62.75	71.46	2.036
Low density lipoprotein	160.28	156.24	159.25	148.37	4.792
Triglycerides	149.37 <sup>a</sup>	141.29 <sup>a</sup>	125.74 <sup>b</sup>	126.15 <sup>b</sup>	3.035

Means with a common superscript within a row are significantly not different at  $P < 0.05$ .

Poultry productivity is influenced by several factors, namely the age of the chicken, ambient temperature, strain, and the nutritional content of the feed (Sodak 2011; Nupur et al. 2023). The average feed conversion value in this study indicated that probiotic supplementation in the ration tended to increase the ration conversion value. Jannah et al. (2022) reported that increasing the feed conversion value in chickens given the probiotic *Bacillus sp.* enabled the digestibility of feed ingredients to be of a higher quality (Haque et al. 2021).

Beta-carotene plays a role in increasing host immunity, preventing acute respiratory infections, playing a role in the process of epithelialization of digestive cells, and also in the proliferation of intestinal mucosal cells (Çalışlar 2019). Probiotic microbes in the digestive tract of poultry can create an acidic environment, thereby spurring the growth of lactic acid bacteria, otherwise suppressing the growth of pathogenic bacteria, so that nutrient absorption can be optimal (Purwati et al. 2015).

Biofeed-H-lysine contains the probiotic *Saccharomyces spp.* which can eliminate *Salmonella* colonization and enhance chicken intestinal immunity (Chen et al. 2012; Yu et al. 2012). Chang et al. (2019) reported that feed supplementation with multi-strain probiotics improved the gut microbiota of chickens and induced different cytokine expression patterns in *Salmonella* infection. According to Kogut and Arsenault (2015), *Salmonella* infection can reduce growth performance and cause dysbacteriosis which results in huge financial losses in the poultry industry.

The total blood serum cholesterol and triglycerides of chickens decreased with the presence of biofeed-H-lysine in the ration. Probiotics as feed additives have been reported to improve nutrient digestibility, growth performance, and the balance of microflora in the intestinal tract. The combination of phytogenic additives with probiotics in feed significantly increases nutrient intake, feed efficiency, and quail health. These results are

supported by Hidayat et al. (2021) who reported that jejunal villi height increased by administering a diet of probiotics, phytobiotics and their combinations. The activity of probiotics and phytobiotics in the intestine can reduce the population of pathogenic bacteria in it, so that these conditions have an impact on increasing the growth and development of intestinal villi (Hedayati and Manafi 2018).

## Conclusion

It can be concluded that there was an increase in the performance of male Bali ducklings fed with the addition of 2-3% Biofeed-H-lysine from 2-10 weeks of age, especially in weight gain and feed efficiency. Conversely, there was a decrease in serum cholesterol and triglyceride levels and total pathogenic bacteria in the intestine.

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## Authors' contributions

All authors (IGNGB, NWS, AAPPW, DPMAC, EP and IMN) were actively involved in the research and writing of this article.

## Conflict of interest

All authors of the manuscript have no conflict of interests to declare.

## REFERENCES

- Ajjiah N, Tjandra BC, Hamidah U, Widyarani and Sintawardani N, 2019. Utilization of tofu waste water as a cultivation medium for *Chlorella vulgaris* and *Arthrospira platensis*. The 4<sup>th</sup> International Symposium on Green Technology for Value Chains 2019, IOP Conf. Series: Earth and Environmental

- Science 483 012027 IOP Publishing <https://doi.org/10.1088/1755-1315/483/1/012027>
- Bidura IGNG, Siti NW, Wibawa AAPP, Puspani E and Candrawati DPMA, 2023. Improving the quality of Tofu waste by mixing it with carrots and probiotics as a feed source of probiotics and  $\beta$ -carotene. *International Journal of Veterinary Science* 12 (3): 407-413. <https://doi.org/10.47278/journal.ijvs/2022.213>
- Batool T, Farooq S, Roohi N and Mahmud A, 2021. Comparative evaluation of body conformation traits in native Aseel chicken fed under different dietary lysine regimens. *The Journal of Animal & Plant Sciences* 31(2): 416-422. <https://doi.org/10.36899/JAPS.2021.2.0230>
- Çalişlar S, 2019. The important of beta-carotene on poultry nutrition. *Selcuk Journal of Agriculture and Food Sciences* 33(3): 252-259.
- Chang CH, Teng PY, Lee TT and Yu B, 2019. The effects of the supplementation of multi-strain probiotics on intestinal microbiota, metabolites and inflammation of young SPF chickens challenged with *Salmonella emicel* subsp. *Animal Science Journal* 90: 737-46. <https://doi.org/10.1111/asi.13205>
- Chen CY, Tsen HY, Lin CL, Yu B and Chen CS, 2012. Oral administration of a combination of select lactic acid bacteria strains to reduce the *Salmonella* invasion and inflammation of broiler chicks. *Poultry Science* 91: 2139-2147. <https://doi.org/10.3382/ps.2012-02237>
- Faisal M, Gani A, Mulana F and Daimon H, 2016. Treatment and utilization of industrial tofu waste in Indonesia. *Asian Journal of Chemistry* 28(3): 501-507. <http://dx.doi.org/10.14233/ajchem.2016.19372>
- Haque MA, Quan H, Zuo Z, Khan A, Siddique N and He C, 2021. Pathogenicity of feed-borne *Bacillus cereus* and its implication on food safety. *Agrobiological Records* 3: 1-16. <https://doi.org/10.47278/journal.abr/2020.015>
- Hedayati M and Manafi M, 2018. Evaluation of an herbal compound, a commercial probiotic, and an antibiotic growth promoter on the performance, intestinal bacterial population, antibody titers, and morphology of the jejunum and ileum of broilers. *Brazilian Journal of Poultry Science* 20(2): 305-316. <https://doi.org/10.1590/1806-9061-2017-0639>
- Hidayat R, Yuniarto VD, Sukanto B and Sugiharto S, 2021. Effect of dietary supplementation of probiotic, phytobiotics or their combination on performance, blood indices and jejunal morphology of laying hens during post peak production. *Online Journal of Animal and Feed Research* 11(1): 08-12. <https://dx.doi.org/10.51227/ojaf.2021.2>
- Jannah SL, Lamid M, Sukmanadi M, Arif MAA, Chusniati S, Hamid IS and Solfaine R, 2022. Potential of giving probiotics to increase body weight, consumption, and feed conversion of laying hens in the pre layer phase. *Media Kedokteran Hewan* 33(2): 96-104. <https://doi.org/10.20473/mkh.v33i2.2022.96-104>
- Jiang Q, Yang F, Gao X and Wu X, 2021. Lysine supplementation improves nutrients digestion, growth performance and liver function of female blue foxes (*Alopex Lagopus*) in growing phase. *Journal of Animal & Plant Sciences* 31(6): 1575-1581. <https://doi.org/10.36899/JAPS.2021.6.0361>
- Kalita R, Pegu A and Baruah C, 2023. Prospects of probiotics and fish growth promoting bacteria in aquaculture: a review. *International Journal of Agriculture and Biosciences* 12(4): 234-244. <https://doi.org/10.47278/journal.ijab/2023.070>
- Kogut MH and Arsenault RJ, 2015. A role for the non-canonical Wnt-beta-catenin and TGF-beta signaling pathways in the induction of tolerance during the establishment of a *Salmonella emicel* serovar enteritidis persistent cecal infection in chickens. *Frontiers in Veterinary Science* 2: 33. <https://doi.org/10.3389/fvets.2015.00033>
- Liao SF, Wang T and Regmi N, 2015. Lysine nutrition in swine and the related monogastric animals: muscle protein biosynthesis and beyond. *SpringerPlus* 4: 147. <https://doi.org/10.1186/s40064-015-0927-5>
- Lieberman A and Burchard R, 1980. Enzymatic method to determined cholesterol. *The New England Journal of Medicine* 271: 915-924.
- Mulia DS, Yulyanti E, Maryanto H and Purbomartono C, 2015. Quality improvement of Tofu waste as the raw material of fish feed with fermentation of *Rhizopus oligosporus*. *Sainteks XII(1)*: 10-20. <https://core.ac.uk/download/pdf/234098605.pdf>
- Nurhayati, Berliana and Nelwida, 2019. Protein efficiency of broiler chicken fed fermented waste tofu with *Saccharomyces cerevisiae*. *Jurnal Ilmiah Ilmu-Ilmu Peternakan* 22(2): 95-106. <https://doi.org/10.22437/jiip.v22i2.6725>
- Nupur MN, Afroz F, Hossain MK, Harun-ur-Rashid SM, Rahman MG, Kamruzzaman M, Ferdous KA and Haque MA, 2023. Prevalence of potential zoonotic bacterial pathogens isolated from household pet birds and their antimicrobial profile in northern Bangladesh. *Agrobiological Records* 11: 28-38. <https://doi.org/10.47278/journal.abr/2023.005>
- Priastoto D, Kurtini T and Sumardi, 2016. The effect of giving probiotics from local microbes on the performance of laying hens. *Jurnal Ilmiah Peternakan Terpadu* 4(1): 80-85. <http://dx.doi.org/10.23960/jipt.v4i1.p%25p>
- Purwati DMA, Djaelani and Yuniwanti EYW, 2015. Egg yolk index (IKT), haugh unit (HU) and egg weight in various local ducks in Central Java. *Jurnal Biologi* 4(2): 1-9.
- Saferi AAS, Emtiazi G, Hajrasulih S and Shariatmadari H, 2005. Biodegradation of some agricultural residues by fungi in agitated submerged cultures. *African Journal of Biotechnology* 4(10): 1058-1061. <https://doi.org/10.5897/AJB2005.000-3210>
- Sari NMLP, Bidura IGNG and Siti NW, 2016. Effect of rations containing tofu dregs fermented with yeast *Saccharomyces sp.* on the physical composition of broiler carcasses aged 6 weeks. *Peternakan Tropika* 4(1): 170-183.
- Soccol CR, Vandenberghe LP, Spier MR, Medeiros AB, Yamaguishi, CT, Lindner JD, Pandey A and Thomaz-Soccol V, 2010. The potential of probiotics: a review. *Food Technology and Biotechnology* 48: 413-434.
- Sodak JF, 2011. Physical and Chemical Characteristics of Arabic Chicken Eggs on Two Livestock Farms in Tulungagung Regency, East Java. Thesis. Faculty of Animal Husbandry. Bogor Agricultural Institute, Bogor, Indonesia.
- Sudatri NW, 2021. Identification of active compounds of turmeric rhizome (*Curcuma domestic* Val.) and tamarind fruit (*Tamarindus indica* L.) and their implementation to improve health performance and broiler production. Dissertation, Doctoral Program, Faculty of Animal Husbandry, Udayana University, Denpasar, Indonesia. 2021.
- Susalam MK, Harentis, Marlida Y, Jamsari and Ardani LR, 2024. The effect of probiotics consortium isolated from fermented fish (Budu) on broiler performances and meat quality. *International Journal of Veterinary Science* 13(1): 100-107. <https://doi.org/10.47278/journal.ijvs/2023.066>
- Yu Q, Zhu L, Wang Z, Li P and Yang Q, 2012. *Lactobacillus delbrueckii ssp. Lactis* R4 prevents *Salmonella typhimurium* SL1344-induced damage to tight junctions and adherens junctions. *Journal of Microbiology* 50: 613-617. <https://doi.org/10.1007/s12275-012-1596-5>
- Zeng PL, Yan HC, Wang XQ, Zhang CM, Zhu C, Shu G and Jiang QY, 2013. Effects of dietary lysine levels on apparent nutrient digestibility and serum amino acid absorption mode in growing pigs. *Asian-Australasian Journal of Animal Sciences* 26(7): 1003-1011. <https://doi.org/10.5713/ajas.2012.12555>
- Zurmiati ME, Mahata MH, Abbas and Wizna, 2014. The application of probiotic on duck. *Jurnal Peternakan Indonesia* 16(2): 134-144; <https://doi.org/10.25077/jpi.16.2.134-144.2014>