Vol. 14(1): 358-370, March 2026 DOI: https://doi.org/10.23960/jipt.v14i1.p358-370

Jurnal Ilmiah Peternakan Terpadu

Journal homepage: https://jurnal.fp.unila.ac.id/index.php/JIPT

p-ISSN: 2303-1956
e-ISSN: 2614-0497

Betaine Supplementation Enhances the Survival Rate of Japanese Quail by Modulating Intestinal Development and Reducing the Growth of Pathogenic Bacteria

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ARTICLE HISTORY:
Submitted: 7 January 2026
Revised: 12 February 2026
Accepted: 13 February 2026
Published: 1 March 2026

KATA KUNCI:
Analisis metagenomik
Betaine
Bakteri patogen
Puyuh
Tingkat kelangsungan hidup

ABSTRAK

Penelitian ini mengkaji pengaruh suplementasi betain terhadap perkembangan usus, komposisi mikrobiota, dan survival rate puyuh (Coturnix japonica) yang dipelihara pada lingkungan tropis. Penelitian menggunakan 300 ekor (berumur 9 minggu; 140±5,79g) dialokasikan ke dalam dua periode, yaitu periode perlakuan yang meliputi pemberian pakan basal (Kontrol) dan Kontrol + suplementasi betain 0,12% (BS), serta periode pascaperlakuan yang terdiri atas pakan Kontrol dan pakan BS yang sudah tidak disuplementasi betain (BW). Hasil penelitian menunjukkan bahwa BS meningkatkan panjang jejunum dan ileum dibandingkan Control (p<0,05). BS secara signifikan meningkatkan survival rate (p<0,05) dibandingkan Control (95,93% vs. 91,76%). Selama periode penghentian, survival rate perlakuan BW (85,25%) tetap lebih tinggi dibandingkan Control (79,43%). Analisis mikrobiologi menunjukkan bahwa suplementasi betain signifikan menurunkan kelimpahan relatif C. perfringens (p<0,05), dengan nilai $4{,}08\times10^{-1}$ (Control), dan $1{,}13\times10^{-3}$ (BS). Tetapi, betain tidak mempengaruhi E. coli, C. spiroforme, dan S. epidermidis. Disimpulkan bahwa suplementasi betain meningkatkan survival rate puyuh di lingkungan tropis melalui peningkatan perkembangan usus dan penurunan bakteri patogen tertentu pada saluran pencernaan.

KEYWORDS:
Betaine
Metagenomic analysis
Pathogenic bacteria
Quail
Survival rate

ABSTRACT

This study investigated the effects of betaine supplementation on the intestinal development, microbiota composition, and survival rate of Japanese quail (*Coturnix japonica*) raised in a tropical environment. The experiment was conducted using 300 birds (9-week-old; 140±5.79g) were allocated into two periods: the treatment period, which included a basal diet (Control) and a diet with 0.12% betaine (BS), and the post-treatment period, comprising the Control group and a group with betaine withdrawn (BW). Results indicated that BS significantly increased the lengths of the jejunum and ileum compared to the control ($p<0.05$). Further, BS group exhibiting a higher survival rate ($p<0.05$) compared to the control (95.93% vs. 91.76%). During the post-treatment period, a higher survival rate was observed in the BW group (85.25%) compared to the control group (79.43%). Microbiological analysis revealed that BS led to a notable reduction in the relative abundance of *C. perfringens* ($p<0.05$), with values: 4.08×10^{-1} (Control), 1.13×10^{-3} (BS). Betaine had no significant effect on the abundance of *E. coli*, *C. spiroforme* or *S. epidermidis*. In conclusion, BS enhances the survival rates of

Japanese quail in tropical climates by promoting intestinal development and inhibiting the growth of specific pathogenic gut bacteria.

1. Introduction

Poultry survival and livability are strongly influenced by environmental conditions, particularly ambient temperature and humidity (Kumar et al., 2021). Heat stress is one of the most detrimental stressors, reducing production efficiency and increasing mortality by impairing thermoregulation, digestion, nutrient metabolism, and immune function (Brossi et al., 2018; Rostagno, 2020). Although these physiological responses aid short-term heat dissipation, they restrict feed intake, compromise growth and egg production, and weaken immune defenses, increasing disease susceptibility and mortality (Ahmad et al., 2022). Heat stress also disrupts intestinal integrity by damaging epithelial tight junctions, increasing gut permeability, and promoting bacterial and endotoxin translocation, which triggers systemic inflammation and immune dysfunction, ultimately reducing survivability (Zmrhal et al., 2023). Nutritional strategies are therefore considered practical and cost-effective approaches to mitigate heat stress (Kpomasse et al., 2021). Among dietary additives, betaine has attracted attention due to its dual role as an organic osmolyte and methyl-group donor (Liu et al., 2019; Ratriyanto and Mosenthin, 2018). By supporting methionine regeneration and amino acid metabolism, betaine reduces metabolic stress during heat exposure (Masykur et al., 2021). In addition, betaine protects intestinal integrity by maintaining osmotic balance and epithelial structure, thereby limiting inflammation, improving nutrient absorption, and supporting poultry health and survival rate (Pradista et al., 2022).

Gut health is closely linked to the composition and stability of the intestinal microbiota. Heat stress often disrupts gut integrity, leading to dysbiosis, which is characterized by an overgrowth of pathogenic bacteria and a decline in beneficial microbial populations (Zheng et al., 2021). Such microbial imbalances have been linked to weakened immune responses, intestinal inflammation, and a decreased survival rate in poultry. Advances in next-generation sequencing and metagenomics now allow comprehensive characterization of gut microbial communities, improving understanding of host–microbiota interactions and their functional implications (Soumeh et al., 2021).

While numerous studies have investigated the effects of betaine on growth performance, stress tolerance, or gut morphology in broilers and laying hens, these investigations have largely focused on single outcome variables or short-term physiological responses. Moreover, studies integrating intestinal development, gut microbiota composition, and survival rate within a single experimental framework remain scarce, particularly in laying Japanese quail reared under tropical environmental conditions. This limitation restricts a holistic understanding of how nutritional interventions modulate gut–host interactions and survival rate under heat stress. Therefore, this study aimed to examine the effects of dietary betaine supplementation on intestinal development, gut microbiota, and survival rate in laying Japanese quail reared in a tropical environment, providing new insights into strategies for improving quail survivability in tropical production systems.

2. Material and Methods

All procedures involving animals adhered to the institutional guidelines for the care and use of animals in research. Ethical approval was obtained from the Animal Ethics Committee, Universitas Sebelas Maret in Surakarta, Indonesia (No. 19/UN27.20/2023).

2.1. Animal and Experimental Design

This study involved 300 quails (*Coturnix japonica*), aged 9 weeks and body weight 140.00 ± 5.79 grams, and was conducted using a completely randomized design consisted of two dietary treatments, with six replicates per treatment, and 25 quails per replicate, resulting in 150 birds per period. The experimental unit was the cage, with each cage housing 25 quails. The birds were allocated into two periods: the treatment period, which included a basal diet (Control) and a diet with 0.12% betaine (BS), and the post-treatment period, comprising the Control group and a group with betaine withdrawn (BW). These periods represented sequential biological observation phases rather than experimental factors within the design. The basal diet was formulated according to the nutritional requirements of quails (NRC, 1994) as shown in Table 1. The inclusion level of 0.12% betaine was selected based on previous poultry studies reporting optimal physiological and performance responses at inclusion levels ranging from 0.10 to 0.15%, particularly under heat stress conditions (Wang et al., 2025). Feed and water were provided *ad libitum*.

The study was conducted in a tropical environment, with ambient temperatures ranging from 26 to 32.4 °C.

Table 1. Composition and nutritional content of the basal diet.

Ingredients (%)	Composition	Nutrient contents	Value
Corn	44.70	Metabolizable energy (kcal/kg)	2,800
Rice bran	7.82	Crude protein (%)	20.00
Wheat	5.00	Ether extracts (%)	4.55
Soybean meal	25.35	Crude fiber (%)	4.48
Fishmeal	7.00	Crude ash (%)	6.36
Coconut oil	2.60	Calcium (%)	3.35
DL-methionine	0.03	Available phosphorus (%)	0.46
Dicalcium phosphate	0.60	Lysine (%)	1.17
Limestone	6.30	Methionine (%)	0.41
Premix*	0.25	Cystine (%)	0.32
NaCl	0.35	Methionine + cystine (%)	0.73

Note: * The premix products which contained per kilogram: 12,000 IU vitamin A; 2,000 IU vitamin D; 8 IU vitamin E; 2 mg vitamin K; 2 mg vitamin B1; 5 mg vitamin B2; 0.5 mg vitamin B6; 12 µg vitamin B12; 12 mg vitamin C; 6 mg calcium *D*-pantothenate; 40 mg niacin; 10 mg choline chloride; 30 mg methionine; 30 mg lysine; 120 mg manganese; 20 mg iron; 0.2 iodine; 100 mg zinc; 0.2 mg cobalt; 4 mg copper; 10 mg antioxidant (santoquin)

2.2. Environmental Condition

During the study, average ambient temperatures were 26.28±1.92 °C in the morning, 32.44±2.10°C during the day, and 29.57±1.85 °C in the evening, indicating clear diurnal temperature fluctuations, with the highest temperatures occurring during the daytime. Based on Marai et al. (2001), a temperature–humidity index (THI) above 26 indicates heat stress in poultry; the mean THI during the study was 27.91±2.55, confirming that the quails were under heat stress.

2.3. Data Collection

The parameters of the digestive tract measured in this study included the lengths of the duodenum, jejunum, and ileum. Measurements of digestive tract length were performed at the end of each observation period, at weeks 18 and 22, each using three individuals from each replicate with similar body weights. The survival rate was calculated by dividing the total number of quails at the end of the observation period by the total number of quails at the beginning of the observation period. For all measured variables, data were first averaged per replicate (cage) prior to statistical analysis, as the cage was defined as the experimental unit. Bacterial diversity levels were assessed using samples of cecal content collected during surgery. Quails were slaughtered by severing the carotid arteries, after which the abdominal cavity was immediately opened under aseptic conditions. The ceca were carefully excised, and their contents were gently collected into sterile tubes. A total of nine samples were subjected to next-generation sequencing, comprising three samples from the Control, BS, and BW groups, respectively. These samples were preserved in RNA later to prevent degradation. The extraction and sequencing of the 16S rRNA gene region V3-V4 were performed at PT Genetika Science Indonesia using the CTAB/SDS extraction method. Amplification, purification, and quality control steps were carried out to produce clean tags. Quality control procedures were implemented using the Qiime quality control procedure Version 1.7.0. Bacterial species annotation was performed based on the SILVA database with a similarity threshold of 0.8--1 (Edgar et al. 2011).

2.4. Statistical Analysis

Two independent samples t-tests were used to compare the Control and BS groups during the treatment period and the Control and BW groups during the post-treatment period for statistically significant differences. Data from the treatment and post-treatment periods were analyzed independently, and no direct statistical comparisons were performed between periods. Alpha diversity indices, including Shannon, Simpson, and Chao1, were analyzed using one-way analysis of variance (ANOVA) after data normality and homogeneity of variance were confirmed. When a significant treatment effect was detected, mean comparisons were performed using Tukey's post hoc test. Differences were considered statistically significant at $p<0.05$. Correlation analysis between survival rate and selected bacterial taxa was performed using Pearson's correlation coefficient (r), as the data met assumptions of normality. Correlation analyses were conducted to assess the strength and direction of associations between variables. Principal Component Analysis (PCA) was conducted as an exploratory multivariate approach to visualize patterns and similarities in cecal bacterial community structure among treatments. PCA results were interpreted descriptively to illustrate clustering tendencies and were not used to infer statistical significance between groups. The data analysis and visualization for this study were carried out using R software version 4.2.2, with assistance from packages such as '*FactoMineR*,' '*factoextra*,' and '*ggplot2*' (R Core Team, 2021).

3. Results and Discussion

3.1. Digestive Tract Development and Survival Rate

Dietary betaine supplementation increased jejunal and ileal lengths during the supplementation period (BS) compared with the Control group ($p<0.05$; Table 2). A similar increase in intestinal length was also observed during the post-treatment period (BW) relative to the Control group ($p<0.05$). Although no direct statistical comparisons were performed between the treatment and post-treatment periods, the persistence of increased intestinal length following betaine withdrawal suggests a potential residual effect of prior supplementation. Consistent with previous reports, betaine has been shown to enhance small intestinal length, improve mucosal architecture, and thereby promote more efficient nutrient absorption (Song et al., 2021). These improvements are attributable to the osmolytic properties of betaine, which help maintain mucosal integrity under physiological stress conditions. As a methyl group donor, betaine supports protein and energy metabolism, facilitating intestinal epithelial cell proliferation, as reflected by increased intestinal length and villus height observed (Liu et al., 2019; Ratriyanto and Mosenthin, 2018). Moreover, betaine's role in osmotic regulation enables the intestine to withstand elevated luminal osmotic pressure caused by unabsorbed nutrients, thereby supporting optimal intestinal growth and development (Ratriyanto and Prastowo, 2019).

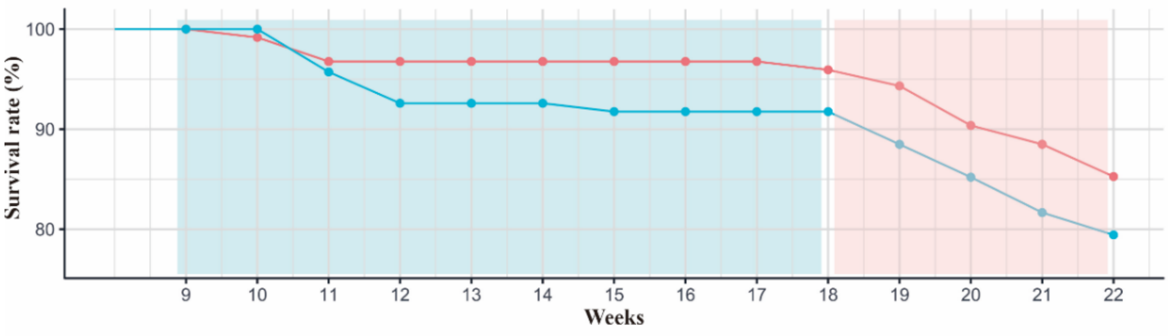
Betaine increased the quail survival rate by 4.54% between weeks 9 and 18 compared to the Control ($p<0.05$; Figure 1). In week 19, betaine supplementation was withdrawn to evaluate its post-supplementation effects. The results for weeks 19 to 22 indicate a decreasing trend in survival rate for both the Control and BW treatments. However, the BW treatment still maintains higher survival rates compared to the Control (Table 2 and Figure 1). This finding aligns with previous reports indicating that betaine enhances stress tolerance and overall physiological resilience in poultry, particularly under challenging environmental conditions such as heat stress (Rostagno, 2020).

Betaine contributes to improved poultry survival by functioning as both an osmolyte and a methyl donor, thereby supporting cellular hydration and protein integrity and enhancing the resistance of intestinal cells to osmotic and heat-related stress (Ratriyanto and Mosenthin, 2018). This improved cellular integrity and gut function enhance nutrient absorption and immune competence, leading to higher survival rates (Santos et al., 2018). Following the post-treatment period, both the Control and BW groups exhibited a decline in survival rate between weeks 19 and 22, apparently due to cumulative stress and age-related vulnerabilities (Brossi et al., 2018). However, birds that had received betaine in the previous period (BW group) maintained a higher survival rate than the Control group, suggesting a carry-over effect. The relatively higher survival observed in the BW group may reflect a residual benefit of prior betaine supplementation when compared with the Control group within the same period, a pattern that has also been reported in broilers and laying hens exposed to betaine under stress conditions (Wahid et al., 2023). The sustained improvement in survival rate after post-treatment suggests that early dietary intervention may lead to long-term improvements in gut integrity, metabolic efficiency, and immune response. These findings underscore the importance of betaine supplementation in enhancing overall flock resilience.

Table 2. Digestive tract length and survival rate in Japanese quail

Treatments	Body Weight (g)	Duodenum (cm)	Jejunum (cm)	Ileum (cm)	Survival rate (%)
Treatment period (week 9 – 18)					
Control	140.00±5.79	11.76±1.16	25.04±1.86	16.56±1.63	91.76±0.75
BS	142.40±5.82	12.96±1.93	29.99±3.27	19.84±1.29	95.93±0.09
P-value	0.532	0.267	0.019	0.008	<0.001
Post-treatment period (week 19 – 22)					
Control	156.30±4.06	11.24±1.47	25.10±1.07	15.56±1.19	79.43±3.41
BW	155.20±3.47	10.78±1.18	29.86±1.60	17.38±0.79	85.26±2.55
P-value	0.657	0.600	0.001	0.021	0.017

Note: p-value lower than 0.05 within the same column indicates a significant difference; a basal diet (Control); 0.12% betaine supplement diet (BS); and BS – 0.12% betaine supplement diet (BW).



Note: The blue area indicates the treatment period with the blue line (Control) as basal diet and the red line (BS) as 0.12% betaine supplement diet; the red area indicates the post-treatment period with the blue line (Control) as basal treatment and the red line (BW) as B - 0.12% betaine diet)

Figure 1. The survival rate curve of Japanese quail (week 9–22)

3.2. Cecal Bacterial Profiles

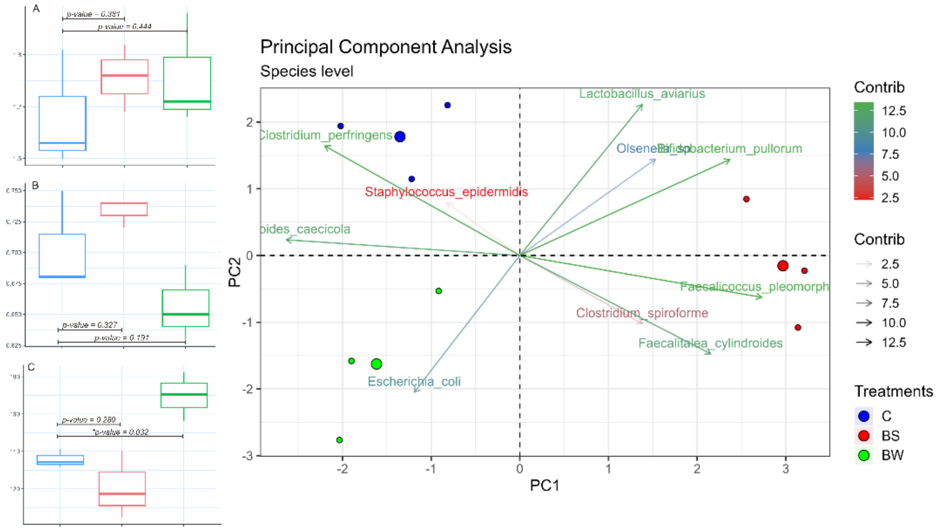
Based on the results of the alpha diversity indices (Shannon and Simpson), the bacterial diversity levels in the ceca of quails given betaine (BS) showed a trend to be higher, although no significant differences were observed compared to the Control group (Figures 2A and 2B). After discontinuation of betaine supplementation (BW), bacterial diversity levels exhibited a decreasing pattern, with no significant differences compared to the Control for both the Shannon and Simpson indices ($p>0.05$; Figure 2A, 2B). Chao1 index, which measures bacterial abundance for each treatment, revealed that the bacterial richness level in the BS group appeared to be slightly lower, with no significant difference compared to the Control ($p>0.05$; Figure 2C). However, there was a significant increase in bacterial richness for the BW group compared to the Control ($p<0.05$; Figure 2C). This increase in richness suggests that the BW group experienced a rise in the abundance of specific bacterial species. Correlation analysis between survival rate and selected bacterial taxa was performed using Pearson's correlation coefficient (r), as the data met assumptions of normality. Correlation analyses were conducted to assess the strength and direction of associations between variables. PCA was used as an exploratory tool to visualize patterns in cecal microbial community composition. The results of the PCA (Figure 2) indicate that data points within the same treatment group are closely clustered, while data points from different treatment groups are more distinctly separated. In the BS group, beneficial bacterial species such as *Bifidobacterium pullorum*, *Lactobacillus aviarius*, and *Faecalicoccus pleomorph* were predominant. These bacterial species influenced by betaine supplementation contribute to the fermentation process that produces short-chain fatty acids (SCFAs). In contrast, the Control and BW groups exhibited different outcomes, with dominant species including pathogenic bacteria such as *Clostridium perfringens*, *Escherichia coli*, and *Staphylococcus epidermidis*. Betaine serves as an osmoprotectant and has been shown to enhance the physiological conditions of the mucosal lining in the digestive tract, thereby optimizing the digestive system in poultry (Pradista et al., 2021). Additionally, the improved digestive efficiency resulting from betaine supplementation creates a more favorable environment for beneficial bacteria to thrive by providing them with essential nutrients (Wang et al., 2025). In the BS group, the bacteria that produce SCFA contribute to an acidic environment, which helps combat pathogenic bacteria (Józefiak et al., 2004). During the treatment period, the abundance of *C. perfringens* was significantly reduced in the BS group compared with the Control group ($p<0.05$; Table 3).

Table 3. Relative abundance of pathogenic bacteria at the species level in the cecum of Japanese quail

Treatments	Relative abundance (%)			
	C. perfringens	*E. coli*	*C. spiroforme*	*S. epidermidis*
Treatment period (week 9 - 18)				
Control	4.08×10^{-1}	1.85×10^{-3}	2.40×10^{-3}	8.57×10^{-2}
BS	1.13×10^{-3}	2.56×10^{-3}	3.70×10^{-2}	2.10×10^{-2}
P-value	0.007	0.793	0.397	0.524
Post-treatment period (week 19 - 22)				
Control	4.08×10^{-1}	1.85×10^{-3}	2.40×10^{-3}	8.57×10^{-2}
BW	1.96×10^{-1}	2.75×10^{-2}	1.16×10^{-2}	2.12×10^{-2}
P-value	0.0138	0.265	0.183	0.522

Note: p-value <0.05 within the same column indicates a significant difference; a basal diet (Control); 0.12% betaine supplement diet (BS); and BS – 0.12% betaine supplement diet (BW).

In the BW group, the levels of *C. perfringens* showed an increasing trend but remained significantly lower than in the Control group ($p<0.05$). *C. perfringens*, a Gram-positive bacterium, produces alpha toxins that can damage the plasma membranes through the action of phospholipase C (Forti et al., 2020). These findings support the role of betaine as an osmoprotectant, which helps minimize membrane damage. This is consistent with the better survival rates observed in the BS group compared to the Control group. The bacterial species *S. epidermidis* did not exhibit significant differences in the treatment and post-treatment periods. The role of *S. epidermidis* has not been extensively reported, but it is indicated to play a role in producing non-aggressive toxins (Otto, 2009). Similarly, *C. spiroforme* and *E. coli* did not show significant differences between the Control and BS groups ($p>0.05$; Table 3). *E. coli* is a Gram-negative pathogenic bacterium that causes infections in the digestive tract. *E. coli* is a common bacterium found in the digestive tract of poultry. While many strains are harmless commensals, some can cause significant diseases, particularly avian pathogenic *E. coli*, which leads to *colibacillosis* (Koutsianos et al., 2020). Further research is needed to identify the specific *serotypes of S. epidermidis, C. spiroforme, and E. coli* involved in this role.



Note: A = Shannon index; **B** = Simpson index; **C** = Chao1 index; **blue** as basal diet (Control); **red** as 0.12% betaine supplement diet (BS); and **green** as BS – 0.12% betaine supplement diet (BW); * p-value lower than 0.05 indicated a significant different)

Figure 2. Boxplot of alpha diversity index and principal component analysis

3.3. Correlation Between Microbial Profiles and Variables

According to the correlation heatmap (Figure 3), the survival rate shows a strong negative correlation with both *C. perfringens* (r=−0.66) and *E. coli* (r=−0.63). These results indicate an inverse relationship between survival rate and the abundance of these bacterial taxes, suggesting that higher bacterial loads are associated with lower survival performance. *C. perfringens* is a well-known causative agent of necrotic enteritis in poultry, which can result in significant mortality. Elevated populations of *C. perfringens* in the intestine, particularly in conjunction with high dietary glycine levels, have been linked to increased intestinal lesions and mortality in broiler chickens (Guo et al., 2023). Similarly, *E. coli* is a common intestinal bacterium in poultry, and certain pathogenic strains have been associated with increased mortality, particularly under conditions of intestinal dysbiosis. The negative correlation observed between *E. coli* abundance and survival rate suggests a potential association with compromised gut health, especially when co-occurring with other pathogenic bacteria such as *C. perfringens.* Overall, the correlation analysis highlights associations between bacterial populations and survival outcomes, which may reflect underlying interactions between gut microbial balance and host resilience. Nevertheless, further studies are required to clarify causal relationships between specific bacterial taxa and survival performance in quails.

Figure 3. Heatmap of correlation between microbial profiles and variables

4. Conclusions

Dietary betaine supplementation was associated with increased jejunal and ileal lengths during the treatment period, with comparable intestinal measurements also observed during the post-treatment period. Betaine was associated with a higher quail survival rate under heat stress, although a declining trend was observed after the post-treatment period. The higher survival rate coincided with a reduced cecal abundance of *C. perfringens*, while the populations of *E. coli, C. spiroforme*, and *S. epidermidis* showed no apparent changes across treatments. Correlation analysis revealed an inverse association between survival rate and *C. perfringens* abundance. These findings suggest that betaine supplementation may be associated with improved intestinal development and survival performance in heat-stressed quails, potentially accompanied by shifts in specific bacterial taxa.

Acknowledgements

Partial funding for this work was provided by Universitas Sebelas Maret (FY 2025) through the "*Penguatan Kapasitas Grup Riset (PKGR-UNS) B*" scheme, in accordance with Research Assignment Agreement No. 371/UN27.22/PT.01.03/2025.

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