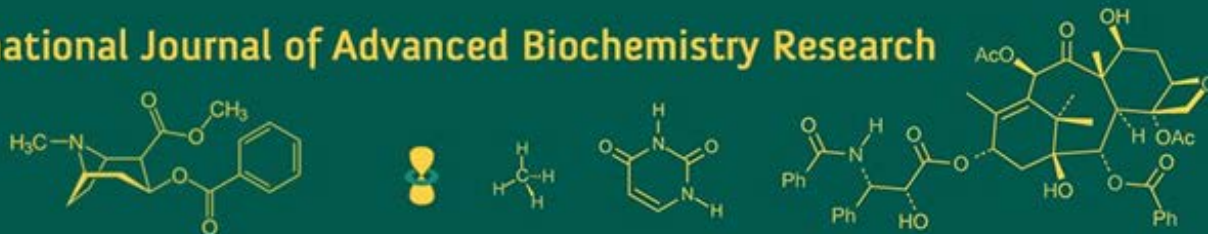


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Effect of dietary α -monolauric acid and *bacillus subtilis* supplementation on growth performance and nutrient digestibility in early-weaned crossbred piglets under heat stress

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Abstract

The present study was conducted to evaluate the effects of dietary supplementation with α -monolauric acid (α -MLA), *Bacillus subtilis* probiotic and their combination on growth performance and nutrient digestibility of early-weaned crossbred piglets reared under heat stress conditions. Twenty-four healthy crossbred piglets (30–35 days old, initial body weight 6.3 ± 0.1 kg) were randomly allotted to 4 dietary treatments with 6 piglets/treatment in a completely randomized design for 92 days. The dietary treatments included: BD - basal diet, BA - basal diet supplemented with α -monolauric acid at 1000 mg/kg, BP - basal diet supplemented with *Bacillus subtilis* probiotic (GutCare®, 2×10^{10} CFU / g) at 500 g/MT and BAP - basal diet supplemented with both α -monolauric acid and probiotic. Growth performance parameters were recorded throughout the trial, while a digestibility trial was conducted during the final 12 days using the double-indicator method. Dietary supplementation significantly ($P < 0.05$) improved growth performance. The combination treatment (BAP) recorded the highest final body weight (27.35 kg), total body weight gain (21.00 kg), and overall average daily gain (230 g/day), followed by BP and BA, compared with the control group. Feed intake and dry matter intake were not significantly affected ($P > 0.05$), although numerically higher values were observed in the supplemented groups. Feed conversion ratio was improved in supplemented piglets, with the lowest value observed in the BAP group (3.47) compared with the control (4.37). Nutrient digestibility was significantly enhanced by supplementation. Piglets receiving the combined treatment exhibited the highest digestibility coefficients for dry matter (85.19%), crude protein (82.71%), ether extract (74.82%), and organic matter (85.08%) ($P < 0.05$), while crude fiber and nitrogen-free extract digestibility showed numerical improvements without statistical significance. The findings demonstrate that dietary supplementation with α -monolauric acid and *Bacillus subtilis*, particularly in combination, enhances growth performance, feed efficiency and nutrient digestibility in earlyweaned crossbred piglets under heat stress conditions. The combined supplementation appears to exert synergistic effects through improved gut health and nutrient utilization, suggesting its potential as an effective nutritional strategy and a promising alternative to antibiotic growth promoters in pig production.

Keywords: α -Monolauric acid, bacillus subtilis, early-weaned piglets, growth performance, nutrient digestibility, heat stress

Introduction

Pig production is an important component of the global livestock industry, with pork accounting for approximately 36% of total meat consumption worldwide (USDA, 2019). Domestic pigs (*Sus domesticus*) are highly valued for their prolific reproductive performance, rapid growth, efficient feed conversion and high dressing percentage, making them one of the most productive meat animals. In India, however, pigs contribute only 4.8% of total meat production, despite a population of 9.06 million animals reported in the 20th Livestock Census, highlighting significant scope for improving pig productivity and meeting the increasing demand for animal protein.

Weaning is one of the most stressful and critical phases in pig production. Early weaning (30–35 days), although beneficial for increasing sow reproductive efficiency by shortening the farrowing interval, exposes piglets to nutritional, environmental, and social stress. These stressors impair intestinal barrier integrity, reduce villus height, decrease digestive enzyme activity, and increase intestinal permeability, predisposing piglets to post-weaning diarrhea, poor nutrient utilization, reduced growth performance, and higher mortality (Smith *et al.*, 2010; Campbell *et al.*, 2013)

[1, 2]. Therefore, nutritional interventions that support gut health and alleviate weaning stress are essential for improving piglet performance. Feed additives such as α -monolauric acid and probiotics have emerged as promising alternatives to antibiotic growth promoters. α -Monolauric acid, a feed additive approved by the U.S. Food and Drug Administration (FDA), possesses broad-spectrum antibacterial and antiviral properties, enhances feed palatability and improves nutrient digestibility by promoting intestinal health (Jiang et al., 2018, Mustafa, 2019, Welch et al., 2020) [3, 4, 5]. Similarly, the probiotic *Bacillus subtilis*, a spore-forming bacterium, enhances gut microbial balance by producing digestive enzymes, stimulating beneficial *Lactobacillus* populations, suppressing enteric pathogens such as *Escherichia coli*, *Salmonella*, *Campylobacter* and *Clostridium spp.*, and improving intestinal health and nutrient utilization (Hosoi et al., 2000, Enthoven and Van der Lee, 2004) [6, 7]. Despite the documented benefits of these additives individually, studies evaluating their combined supplementation, particularly under Indian production conditions and heat stress, remain limited. Therefore, investigating the synergistic effects of α -monolauric acid and *Bacillus subtilis* on growth performance and nutrient digestibility in early-weaned piglets is of considerable scientific and practical importance.

Materials and Methods

A 92-day feeding trial was conducted from 12 April to 12 July 2025 at the Livestock Farm Complex (LFC), College of Veterinary Science, Rajendranagar, Hyderabad, India, to evaluate the effects of α -monolauric acid (α -MLA) and *Bacillus subtilis* probiotic supplementation on the performance and nutrient digestibility of early-weaned crossbred piglets under heat stress conditions. Laboratory analyses were carried out in the Department of Animal Nutrition, College of Veterinary Science, Rajendranagar, Hyderabad.

Experimental Diets

Twenty-four healthy crossbred piglets (30–35 days old) with an average initial body weight of 6.3 ± 0.1 kg were randomly allotted to four dietary treatments in a completely randomized design ($n = 6$). The dietary treatments consisted of: Control (BD)- basal diet without additives, basal diet supplemented with α -monolauric acid at 1000 mg/kg (BA), basal diet supplemented with *Bacillus subtilis* probiotic (GutCare®, 2×10^{10} CFU/g) at 500 g/MT^o (BP) and basal diet supplemented with both α -monolauric acid (1000 mg/kg) and probiotic (500 g/MT) (BAP). The basal diet was formulated according to the nutrient requirements of growing piglets as recommended by ICAR (2024).

Table 1: The four experimental diets include

Diets	Description of diets
BD	Basal diet (without antibiotic or any feed additive)
BA	Basal diet + α - Monolauric acid @ 1000 mg/kg of diet
BP	Basal diet + Probiotic (<i>Bacillus Subtilis</i>) @500 gm/MT of feed
BAP	Basal diet + α - Monolauric acid @ 1000 mg/kg of diet + Probiotic (<i>Bacillus Subtilis</i>) @ 500 gm/MT of feed

Table 2: Ingredient composition of the basal diet.

Ingredient	Parts
Maize	50.5
GNC	38.5
Red gram chunni	4
De-oiled rice bran	2
Dicalcium phosphate	1
Calcite powder	1
Salt	0.5
Mineral mixture	2
L-Lysine HCl	0.30
DL-Methionine	0.20

Each 1.2kg of mineral mixture contains calcium-255g, Phosphorous-127.5g, Magnesium-6.0g, Manganese-1.5g, Iron-1.5g, Iodine-325mg, Copper-4.2g, Zinc-9.6g, Cobalt-150mg, Sulphur-7.2g, Pottassium-100mg, Sodium-6mg, Selenium-10mg, Vitamin A -700000 IU, Vitamin D-7000 IU, Vitamin E-250mg, Nicotinamide-1000mg, Chromium-8mg

Piglets were housed in well-ventilated pens under standard management practices with *ad libitum* access to feed and drinking water throughout the experimental period. Feed was offered twice daily in mash form. During the experimental period, ambient temperatures ranged from 25.5 °C to 46.5 °C, while relative humidity varied between 30% and 66%, indicating heat stress conditions.

Growth Performance Parameters

In this study, growth performance of the piglets was analysed by calculating fortnightly body weight gain, average daily gain and finally total weight gain of all piglets fed experimental diets.

Body Weight Gain

The body weights of the piglets were measured at fortnightly intervals.

Total body weight gain = Final body weight – Initial body weight

Average Daily Gain

$$\text{Averagedailygain} = \frac{\text{Total body weight gain(kg)}}{\text{Total no of days (90)}}$$

Digestion Trail

A digestibility trial was conducted during the final 12 days of the experiment (days 81– 92), comprising a 5-day adaptation period followed by a 7-day collection period. Four piglets from each treatment were selected for digestibility assessment using the double-indicator method. Chromic oxide (Cr_2O_3 ; 3g / day), administered in hydroxypropyl methylcellulose capsules before feeding, served as the external marker. Fresh fecal samples were collected by rectal massage three times daily, pooled for each animal, processed and stored for chemical analysis. Feed and fecal samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), total ash (TA), and nitrogen-free extract (NFE) according to AOAC (2012) [9] procedures. Chromic oxide concentration was determined using the wet digestion method and apparent nutrient digestibility coefficients were calculated using marker ratios. These analyses were used to estimate nutrient digestibility and retention in piglets receiving different dietary treatments.

Digestibility of DM, CP, CF, EE were calculated using internal indicator acid insoluble ash (AIA) using the following formula

$$\text{Digestibility (\%)} = 100 - 100 \times \frac{\frac{\% \text{ Nutrient in feed}}{\% \text{ Nutrient in faeces}} \times \frac{\% \text{ Indicator in faeces}}{\% \text{ Indicator in feed}}}{\frac{\text{Amount of Cr2O3 fed daily (g/d)}}{\text{Marker concentration in faeces (g/g DM)}}}$$

$$\text{Faecal DM output (kg/day)} = \frac{\text{Faecal DM output}}{\text{indigestibility of D}} \times 100 \%$$

$$\text{DM intake (kg/d)} = \frac{\text{Faecal DM output}}{\text{indigestibility of DM}} \times 100 \%$$

Statistical Analysis

Data analysis was performed using the General Linear Model (GLM) procedure in the Statistical Package for Social Sciences (SPSS) version 20. Means were compared using Duncan's multiple range test (Duncan, 1955) [10], with statistical significance set at $P < 0.05$. The experimental data were analyzed under a completely randomized design employing one-way analysis of variance (ANOVA) as described by Snedecor and Cochran (1989).

III. Results and Discussion

The study evaluated the effects of dietary supplementation with α -monolauric acid (α -MLA), *Bacillus subtilis* probiotic, and their combination on growth performance and nutrient digestibility. The findings obtained are presented and discussed in the following sections.

Nutrient Composition of the Diet

The chemical composition of the experimental diets is presented in Table 3. All four dietary treatments were formulated to be isonitrogenous and isocaloric, providing comparable levels of metabolizable energy (3291.6 kcal/kg), crude protein (22.06%), calcium (0.70%), phosphorus (0.60%), lysine (0.95%), and methionine (0.66%), thereby meeting the nutrient requirements of growing piglets.

Table 3: Nutrient composition of the diets fed to the piglets during the experiment

Nutrient	BD	BA	BP	BAP
ME (kcal/kg)	3291.6	3291.6	3291.6	3291.6
CP (%)	22.06	22.06	22.06	22.06
Ca (%)	0.70	0.70	0.70	0.70
P (%)	0.60	0.60	0.60	0.60
Lysine (%)	0.95	0.95	0.95	0.95
Methionine (%)	0.66	0.66	0.66	0.66

Growth Performance

Significant ($P < 0.05$) improvements in fortnightly body weight gain, average daily gain (ADG) and total body weight gain were observed in piglets supplemented with α -monolauric acid, *Bacillus subtilis* and their combination. These improvements are likely attributable to enhanced gut health and improved nutrient digestibility. α -Monolauric acid exerts antimicrobial activity against enteric pathogens, improves intestinal morphology, and enhances nutrient absorption (Jackman *et al.*, 2020, Dahmer *et al.*, 2021, Zeng *et al.*, 2022) [11, 12, 13]. *Bacillus subtilis* promotes beneficial gut microbiota, secretes digestive enzymes, improves nutrient digestibility and supports immune and antioxidant functions (Deng *et al.*, 2020, Mun *et al.*, 2021, Tang *et al.*, 2024) [14, 15, 16]. The greatest response observed in the combined supplementation group suggests a synergistic effect, where reduced pathogen load and improved probiotic colonization together enhance digestive efficiency and growth performance, consistent with previous findings of Jackman *et al.*, 2020 [11]; Tang *et al.*, 2024 [16].

Fortnight Body Weight Gain

Significantly ($P < 0.05$) higher fortnightly body weight gains were observed in piglets supplemented with α -monolauric acid, *Bacillus subtilis*, and their combination (Table 4), with the greatest improvement recorded in the combination group during the second, third, fifth, and sixth fortnights. These findings are consistent with previous studies demonstrating improved body weight gain following supplementation with combinations of organic acids, mediumchain fatty acids, probiotics or yeast-derived products in weaned piglets (Giannenas *et al.*, 2016, Jiao *et al.*, 2023, Liu *et al.*, 2025, Ficagan *et al.*, 2025) [17, 18, 19]. However, some studies have reported no significant effect of glycerol monolaurate, lauric acid or *Bacillus subtilis* supplementation on body weight gain (Cui *et al.*, 2019, Li *et al.*, 2022, Marsha *et al.*, 2024) [20, 21, 22].

These discrepancies may be attributed to differences in additive type and dosage, supplementation period, diet composition, animal age and experimental conditions.

Table 4: Effect of feeding concentrate mixture with supplementation of α -monolauric acid, probiotic and their combination on fortnight bodyweights (kg) of growing cross breed piglets under heat stress conditions.

Body weight	BD	BA	BP	BAP	SEM	P- Value	N
Initial weight (kg)	6.34	6.33	6.34	6.35	0.25	1.00	6
First Fortnight (kg)	7.07	7.80	7.59	7.90	0.13	0.084	6
Second Fortnight (kg)	8.76 ^a	10.75 ^{bc}	9.93 ^b	11.13 ^c	0.26	0.021	6
Third Fortnight (kg)	13.31 ^a	13.85 ^{ab}	14.50 ^b	14.57 ^b	0.14	0.015	6
Fourth fortnight (kg)	15.80	16.42	16.77	17.03	0.27	0.451	6
Fifth Fortnight (kg)	18.08 ^a	20.30 ^{ab}	21.70 ^b	22.85 ^b	0.60	0.016	6
Sixth fortnight (kg)	23.19 ^a	24.81 ^{ab}	26.80 ^b	27.35 ^b	0.44	0.045	6

Total Weight Gain

Significantly ($P < 0.05$) higher total body weight gain was observed in piglets supplemented with α -monolauric acid, *Bacillus subtilis* and their combination (Table 5), with the

greatest improvement recorded in the combined supplementation group. These findings are consistent with reports showing enhanced body weight gain following supplementation with organic acids, medium-chain fatty acids,

probiotics, or their combinations (Thomas *et al.*, 2020, Ngoc *et al.*, 2022, Jiao *et al.*, 2023, Zhao *et al.*, 2024, Ficagan *et al.*, 2025) [23, 18, 24, 25]. However, other studies have reported no significant effects of medium-chain fatty acids, glycerol monolaurate, or *Bacillus subtilis* supplementation on total body weight gain (Cui *et al.*, 2019, Li *et al.*, 2022, Marsha *et al.*,

Table 5: Effect of feeding concentrate mixture with supplementation of α -monolauric acid, probiotic and their combination on total weight gain (kg) of growing cross breed piglets under heat stress conditions.

Treatment	Initial weight (kg)	Final weight (kg)	Total weight gain (kg)
BD	6.34	23.19 ^a	16.85 ^a
BA	6.33	24.81 ^{ab}	18.65 ^{ab}
BP	6.34	26.80 ^b	20.46 ^{bc}
BAP	6.35	27.35 ^b	21.00 ^{bc}
SEM	0.25	0.44	0.48
P-Value	1.00	0.045	0.003
N	6	6	6

^{abc}Means with superscript in a column differ significantly: ($P < 0.05$) P -value: probability value.; SEM: Standard Error Mean N: number of animals per treatment BD: Basal Diet; BA: BD + 1000 mg/kg feed α - monolauric acid; BP: BD + 500gm/metric ton Probiotic; BAP: BD + 1000 mg/kg feed α -

2024, Chiengsorn *et al.*, 2025, Fan *et al.*, 2025) [20, 21, 22, 26, 27]. These variations are likely due to differences in the type and inclusion level of feed additives, supplementation strategies, experimental duration, pig genetics, health status and management conditions.

monolauric acid + 500gm/metric ton Probiotic Significantly ($P < 0.05$) higher average daily gain (ADG) was observed in piglets supplemented with α -monolauric acid, *Bacillus subtilis*, and their combination (Table 6), with the greatest improvement recorded in the combined supplementation group. These findings are in agreement with previous studies reporting significant improvements in ADG following supplementation with medium-chain fatty acids, probiotics, organic acids or their combinations (Suryanarayana *et al.*, 2012, Devi and Kim, 2014, Hanczakowska *et al.*, 2016, Zhang *et al.*, 2016, Lei *et al.*, 2018, da Silva *et al.*, 2021, Ngoc *et al.*, 2022, Jiao *et al.*, 2023, Liu *et al.*, 2025) [28, 18, 19, 24, 29, 30, 31, 32, 20, 33]. In contrast, Li *et al.*, 2022 [21] and Cui *et al.*, 2019 [20] observed no significant effect of glycerol monolaurate or *Bacillus subtilis* supplementation on ADG. Such inconsistencies may be attributed to differences in feed additive type, dosage, duration of supplementation, pig genotype, age, health status, and management or environmental conditions.

Table 6: Effect of feeding concentrate mixture with supplementation of α -monolauric acid, probiotic and their combination on average daily gain (ADG) (g) of growing cross breed piglets under heat stress conditions.

Body weight	BD	BA	BP	BAP	SEM	P - Value	N
First Fortnight (kg)	49.22	97.33	83.22	103.33	13.03	0.505	6
Second Fortnight (kg)	113.00 ^a	165.11 ^{bc}	156.33 ^{bc}	215.67 ^c	12.78	0.030	6
Third Fortnight (kg)	206.20 ^a	229.00 ^{ab}	301.00 ^b	304.80 ^b	14.15	0.013	6
Fourth fortnight (kg)	168.22	171.55	162.11	163.90	9.32	0.987	6
Fifth Fortnight (kg)	252.22 ^a	328.66 ^b	348.00 ^b	340.66 ^b	12.29	0.011	6
Sixth fortnight (kg)	340.11	328.66	350.00	339.00	13.75	0.965	6
Overall average daily gain	190 ^a	210 ^{ab}	220 ^{bc}	230 ^{bc}	0.01	0.003	6

^{abc}Means with superscript in a column differ significantly: ($P < 0.05$) P -value: probability value.; SEM: Standard Error Mean N: number of animals per treatment BD: Basal Diet; BA: BD + 1000 mg/kg feed α - monolauric acid; BP: BD + 500gm/metric ton Probiotic; BAP: BD + 1000 mg/kg feed α -monolauric acid + 500gm/metric ton Probiotic

Feed Intake Parameters

Feed Intake and Dry Matter Intake

No significant ($P > 0.05$) differences were observed in feed intake or dry matter intake among the treatment groups, although piglets receiving the combined supplementation of α -monolauric acid and *Bacillus subtilis* (Table 7) showed numerically higher values. These findings are consistent with several studies reporting that supplementation with medium-chain fatty acids, probiotics, organic acids, or their combinations had no significant effect on feed intake (Devi and Kim, 2014, Zhang *et al.*, 2016, Giannenas *et al.*, 2016, Cui *et al.*, 2019, Ternus *et al.*, 2022, Ngoc *et al.*, 2022, Li *et al.*, 2022, Jiao *et al.*, 2023, Wichasit *et al.*, 2025) [29, 17, 18, 20, 21, 24, 31, 33, 34, 35]. However, Liu *et al.*, 2025 [19], Ficagna *et al.*, 2025 [36] and da Silva *et al.*, 2021 [20] reported significant improvements in feed intake following supplementation with *Bacillus subtilis*, organic acid–yeast cell wall blends, or benzoic acid–probiotic combinations. These variations among studies are likely due to differences in the type and dosage of feed additives, supplementation strategies, experimental duration, animal age

and physiological status, and environmental and management conditions.

Feed Conversion Ratio

Piglets supplemented with α -monolauric acid, *Bacillus subtilis*, and their combination exhibited an improved feed conversion ratio (FCR) compared with the control group, with the greatest improvement observed in the combined supplementation group (Table 7). Despite no significant ($P > 0.005$) differences in feed intake, the lower FCR indicates enhanced nutrient utilization and growth efficiency, likely resulting from improved intestinal health, nutrient digestibility, and gut microbial balance. These findings agree with previous studies reporting improved FCR following supplementation with probiotics, organic acids, medium-chain fatty acids, or their combinations (Giannenas *et al.*, 2016, Marsha *et al.*, 2019, Ternus *et al.*, 2022, Liu *et al.*, 2025, Ficagna *et al.*, 2025) [17, 19, 34, 36]. However, Cui *et al.*, 2019 [20], da Silva *et al.*, 2021 [20] and Li *et al.*, 2022 [21] reported no significant effects of similar feed additives on FCR. Such variations may be attributed to differences in dietary composition, additive type and inclusion level, experimental duration, animal age and health status, and environmental and management conditions.

Table 7: Effect of feeding concentrate mixture with supplementation of α -monolauric acid, probiotic and their combination on feed intake (kg) and feed conversion ratio (kg feed/kg gain) of growing cross breed piglets under heat stress conditions.

Treatment	Dry matter intake (kg)	Feed intake (kg)	Feed Conversion Ratio (kg feed/kg gain)
BD	0.72	0.80	4.37 ^{ab}
BA	0.74	0.81	3.70 ^{ab}
BP	0.74	0.81	3.58 ^a
BAP	0.78	0.82	3.47 ^a
SEM	0.25	0.06	0.13
P-Value	0.99	0.42	0.062
N	6	6	6

^{abc}Means with superscript in a column differ significantly: ($P < 0.05$)

P-value: probability value.; SEM: Standard Error Mean N: number of animals per treatment BD: Basal Diet; BA: BD + 1000 mg/kg feed α - monolauric acid; BP: BD + 500gm/metric ton Probiotic; BAP: BD + 1000 mg/kg feed α - monolauric acid + 500gm/metric ton Probiotic

Significant ($P < 0.05$) improvements in the digestibility of dry matter (DM), crude protein (CP), and ether extract (EE) were observed in piglets supplemented with α -monolauric acid,

Table 8: Effect of feeding concentrate mixture with supplementation of α -monolauric acid, probiotic and their combination on nutrient digestibility (%) of growing cross breed piglets under heat stress conditions

Treatment	Dry matter	Organic matter	Crude protein	Crude Fiber	Ether extract	Nitrogen free extract
BD	78.09 ^a	80.93 ^a	77.83 ^a	35.04	69.72 ^a	66.02
BA	82.05 ^{ab}	83.50 ^{ab}	80.69 ^{ab}	39.50	72.21 ^{ab}	68.02
BP	83.38 ^{ab}	84.22 ^{ab}	80.98 ^{ab}	39.98	72.39 ^{ab}	68.85
BAP	85.19 ^c	85.08 ^{ab}	82.71 ^c	40.03	74.82 ^c	69.56
SEM	0.89	0.41	0.68	1.05	0.50	0.679
P-Value	0.001	0.007	0.025	0.057	0.020	0.242
N	6	6	6	6	6	6

^{abc}Means with superscript in a column differ significantly: ($P < 0.05$)

P-value: probability value.; SEM: Standard Error Mean N: number of animals per treatment BD: Basal Diet; BA: BD + 1000 mg/kg feed α - monolauric acid; BP: BD + 500gm/metric ton Probiotic; BAP: BD + 1000 mg/kg feed α - monolauric acid + 500gm/metric ton Probiotic

IV. Conclusion

Dietary supplementation with α -monolauric acid, *Bacillus subtilis*, and their combination improved the growth performance and nutrient digestibility of early-weaned crossbred piglets under heat stress conditions. The combined supplementation produced the best results, with significantly higher body weight gain, average daily gain, improved feed conversion ratio, and greater digestibility of dry matter, crude protein, ether extract, and organic matter. These findings suggest that the combination of α -monolauric acid (1000 mg/kg feed) and *Bacillus subtilis* probiotic (500 g/metric ton of feed) is an effective nutritional strategy to enhance piglet performance and nutrient utilization, and may serve as a promising alternative to antibiotic growth promoters.

Bacillus subtilis, and their combination (Table 8), whereas crude fiber and nitrogen-free extract digestibility showed only numerical improvements. The enhanced nutrient digestibility is likely due to the complementary effects of α -monolauric acid and *Bacillus subtilis* on intestinal health. α -Monolauric acid improves intestinal morphology, stimulates digestive enzyme secretion, enhances lipid digestion and increases the absorptive surface area, thereby promoting nutrient utilization (Dierick *et al.*, 2003, Awad *et al.*, 2006, Zentek *et al.*, 2012) [37, 38, 39]. *Bacillus subtilis* further enhances digestion by producing extracellular enzymes, improving gut microbial balance and stimulating intestinal epithelial development through short-chain fatty acid production (Hosoi *et al.*, 2000, Wang and Gu, 2010) [6, 40]. These findings are consistent with previous studies demonstrating improved nutrient digestibility following supplementation with medium-chain fatty acids, probiotics, or their combinations (Devi and Kim, 2014, Lei *et al.* 2018, Ngoc *et al.*, 2022, Hu and Kim, 2022, Jiao *et al.*, 2023, Li *et al.*, 2022, Marsha *et al.*, 2024, Liu *et al.*, 2025) [29, 18, 19, 21, 22, 24, 32, 41]. However, some studies reported selective or non-significant effects on certain nutrients, particularly with medium-chain fatty acids alone (Gebhardt *et al.*, 2020; Fan *et al.*, 2025) [42, 27], likely due to differences in additive type, dosage, diet composition and experimental conditions.

References

- Smith F, Clark JE, Overman BL, Tozel CC, Huang JH, Rivier JE, *et al.* Early weaning stress impairs development of mucosal barrier function in the porcine intestine. *American Journal of Physiology-Gastrointestinal and Liver Physiology*. 2010;298(3).
- Campbell JM, Crenshaw JD, Polo J. The biological stress of early weaned piglets. *Journal of animal science and biotechnology*. 2013;4(1):19.
- Jiang Z, Zhao M, Zhang H, Li Y, Liu M, Feng F. Antimicrobial emulsifier-glycerol monolaurate induces metabolic syndrome, gut microbiota dysbiosis, and systemic low grade inflammation in low fat diet fed mice. *Molecular nutrition & food research*. 2018;62(3):1700547.
- Mustafa NG. Biochemical trails associated with different doses of alpha-monolaurin in chicks. *Advances in Animal and Veterinary Science*. 2019;7(3):187-192.
- Welch JL, Xiang J, Okeoma CM, Schlievert PM, Stapleton JT. Glycerol monolaurate, an analogue to a factor secreted by *Lactobacillus*, is virucidal against enveloped viruses, including HIV-1. *Mbio*. 2020;11(3):10-128.
- Hosoi T, Ametani A, Kiuchi K, Kaminogawa S. Improved growth and viability of lactobacilli in the presence of *Bacillus subtilis* (natto), catalase, or subtilisin. *Canadian Journal of Microbiology*. 2000;46(10):892-7.
- Enthoven P, van der Lee A. Compatibility of Calsporin® (preparation of viable spores of *Bacillus subtilis* C-3102) with EU approved coccidiostats, antimicrobials and organic acids in broiler feeds. 2004.
- AOAC. Official Methods of Analysis of AOAC International 19th edition. AOAC, International, Gaithersburg, Maryland, USA; 2012.
- Duncan DB. Multiple range and multiple F tests. *Biometrics*. 1955;11(1):1-42.
- Jackman JA, Hakobyan A, Zakaryan H, Elrod CC. Inhibition of African swine fever virus in liquid and feed by medium-chain fatty acids and glycerol monolaurate. *Journal of animal science and biotechnology*. 2020;11(1):114.

11. Dahmer PL, Jones CK. Evaluating dietary acidifiers as alternatives for conventional feed-based antibiotics in nursery pig diets. *Translational animal science*. 2021;5(2).
12. Zeng X, Yang Y, Wang J, Wang Z, Li J, Yin Y, *et al*. Dietary butyrate, lauric acid and stearic acid improve gut morphology and epithelial cell turnover in weaned piglets. *Animal Nutrition*. 2022;11:276-282.
13. Deng B, Wu J, Li X, Zhang C, Men X, Xu Z. Effects of *Bacillus subtilis* on growth performance, serum parameters, digestive enzyme, intestinal morphology, and colonic microbiota in piglets. *Amb Express*. 2020;10(1):212.
14. Mun D, Kyoung H, Kong M, Ryu S, Jang KB, Baek J, *et al*. Effects of *Bacillus* based probiotics on growth performance, nutrient digestibility, and intestinal health of weaned pigs. *Journal of Animal Science and Technology*. 2021;63(6):1314.
15. Tang X, Zeng Y, Xiong K, Zhong J. *Bacillus* spp. as potential probiotics: promoting piglet growth by improving intestinal health. *Frontiers in Veterinary Science*. 2024;11:1429233.
16. Giannenas I, Doukas D, Karamoutsios A, Tzora A, Bonos E, Skoufos I, *et al*. Effects of *Enterococcus faecium*, mannan oligosaccharide, benzoic acid and their mixture on growth performance, intestinal microbiota, intestinal morphology and blood lymphocyte subpopulations of fattening pigs. *Animal Feed Science and Technology*. 2016;220:159-167.
17. Jiao S, Zheng Z, Zhuang Y, Tang C, Zhang N. Dietary medium-chain fatty acid and *Bacillus* in combination alleviate weaning stress of piglets by regulating intestinal microbiota and barrier function. *Journal of Animal Science*. 2023;101:skac414.
18. Liu Y, Cao L, Yu C, Zhou Q, Li H, Zhang R, *et al*. Dietary supplementation with *Bacillus subtilis* PB6 alleviates diarrhea and improves growth performance and immune function in weaned piglets fed a high-protein diet. *Frontiers in Veterinary Science*. 2025;12:1525354.
19. Cui K, Diao Q, Zhang N. Effects of dietary supplementation with *Bacillus subtilis* and yeast culture on growth performance, nutrient digestibility, serum indices and faeces microbiota of weaned piglets. *Journal of Animal and Feed Sciences*. 2019;28(4):328-336.
20. Li L, Wang H, Zhang N, Zhang T, Ma Y. Effects of α -glycerol monolaurate on intestinal morphology, nutrient digestibility, serum profiles, and gut microbiota in weaned piglets. *Journal of Animal Science*. 2022;100(3):skac046.
21. Marsha GG, Venkateswarlu M, Alexander G, Rao MH, Ekambaram B, Parashuramulu S. Dietary lauric acid supplementation in large white yorkshire cross piglets and its effect on production and health. *Indian Journal of Veterinary and Animal Sciences Research*. 2024;52(6):69-82.
22. Thomas LL, Woodworth JC, Tokach MD, Dritz SS, DeRouchey JM, Goodband RD, *et al*. Evaluation of different blends of medium-chain fatty acids, lactic acid, and monolaurin on nursery pig growth performance. *Translational Animal Science*. 2020;4(2):548-557.
23. Ngoc TTB, Huyen NT, Oanh NC, Dang PK. Effects of probiotics and organic acids and their mixture on nutrient digestibility, growth performance and fecal gas emission in grower-finisher pigs. *Advances in Animal and Veterinary Science*. 2022;10(3):480-487.
24. Zhao J, Xie Z, Zheng M, Tang W, Diao H, Yin H. Dietary complex probiotic supplementation changed the composition of intestinal short-chain fatty acids and improved the average daily gain of weaned piglets. *Frontiers in Veterinary Science*. 2024;11:1424855.
25. Chiengsorn K, Prasongsoo S, Bunchasak C, Kayan A, Poeikhampha T. Dietary MCFAs and lauric acid as antibiotic alternatives: Effects on growth, immunity and gut health in weaned pigs. *Advances in Animal and Veterinary Science*. 2025;13(8):1856-1863.
26. Fan Z, Lei L, Wu X, Xing R, Du P, Wang Z, *et al*. Dietary fatty acids promote gut health in weaned piglets by regulating gut microbiota and immune function. *Frontiers in Microbiology*. 2025;16:1558588.
27. Suryanarayana MVAN, Suresh J, Rajasekhar MV. A study on the associated effect of probiotic and citric acid on the performance of pre-weaned piglets. *Tamil Nadu Journal of Veterinary and Animal Science*. 2012;8(3):126-30.
28. Devi SM, Kim IH. Effect of medium chain fatty acids (MCFA) and probiotic (*Enterococcus faecium*) supplementation on the growth performance, digestibility and blood profiles in weanling pigs. *Veterinarni Medicina*. 2014;59(11).
29. Hanczakowska E, Swiatkiewicz M, Natonek Wisniewska M, Okon K. Medium chain fatty acids (MCFA) and/or probiotic *Enterococcus faecium* as a feed supplement for piglets. *Livestock Science*. 2016;192:1-7.
30. Zhang MS, Sandouk A, Houtman JC. Glycerol Monolaurate (GML) inhibits human T cell signaling and function by disrupting lipid dynamics. *Scientific reports*. 2016;6(1):30225.
31. Lei XJ, Lee SI, Lee KY, Nguyen DH, Kim IH. Effects of a blend of organic acids and medium-chain fatty acids with and without *Enterococcus faecium* on growth performance, nutrient digestibility, blood parameters, and meat quality in finishing pigs. *Canadian journal of animal science*. 2018;98(4):852-859.
32. Zhang ZF, Rolando AV, Kim IH. Effects of benzoic acid, essential oils and *Enterococcus faecium* SF68 on growth performance, nutrient digestibility, blood profiles, faecal microbiota and faecal noxious gas emission in weanling pigs. *Journal of Applied Animal Research*. 2016;44(1):173-179.
33. Ternus EM, Piroca L, Matte F, Dias CP, Callegari MA, Oliveira ER, *et al*. Acidifiers and prebiotics in the diets of nursery-stage piglets as alternatives to antibiotic growth promoters. *Semina: Ciências Agrárias*. 2022;43(5):2221-2236.
34. Wichasit N, Tartrakoon W, Charoensook R, Singanusong R, Looor JJ, Shoji G, *et al*. Effect of Microencapsulated Medium-Chain Fatty Acids, Lignocellulose, and Heat-Killed *Lactobacillus plantarum* L-137 Supplementation on Lactating Sow Performance, and Nutritional and Immunological Parameters in Colostrum. *Veterinary Sciences*. 2025;12(2):134.
35. Ficagna CA, Silva ASD, Rofino RD, Zatti E, Esposito T, Xavier ACH, *et al*. Effects on performance, immunological response and short-chain fatty acid profile in feces of nursery piglets fed with organic acids and yeast wall. *Animals*. 2025;15(7):1051.
36. Dierick NA, Decuyper JA, Degeyter I. The combined use of whole Cuphea seeds containing medium chain fatty acids and an exogenous lipase in piglet nutrition. *Archives of Animal Nutrition*. 2003;57(1):49-63.
37. Awad WA, Bohm J, Razzazi Fazeli E, Ghareeb K, Zentek J. Effect of addition of a probiotic microorganism to broiler

diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens. Poultry Science. 2006;85(6):974-979.

38. Zentek J, Buchheit Renko S, Männer K, Pieper R, Vahjen W. Intestinal concentrations of free and encapsulated dietary medium-chain fatty acids and effects on gastric microbial ecology and bacterial metabolic products in the digestive tract of piglets. Archives of Animal Nutrition. 2012;66(1):14-26.
39. Wang Y, Gu Q. Effect of probiotic on growth performance and digestive enzyme activity of Arbor Acres broilers. Research in Veterinary Science. 2010;89(2):163-167.
40. Hu J, Kim IH. Effect of *Bacillus subtilis* C-3102 spores as a probiotic feed supplement on growth performance, nutrient digestibility, diarrhea score, intestinal microbiota, and excreta odor contents in weanling piglets. Animals. 2022;12(3):316.
41. Gebhardt JT, Thomson KA, Woodworth JC, Dritz SS, Tokach MD, DeRouchey JM, *et al.* Effect of dietary medium-chain fatty acids on nursery pig growth performance, fecal microbial composition, and mitigation properties against porcine epidemic diarrhea virus following storage. Journal of animal science. 2020;98(1):skz358.
42. Snedecor GW, Cochran WG. Statistical Methods. New Delhi: Oxford and IBH Publishing Company; 1980.